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THE

REGIONAL RESEARCH CENTRE

of the British Caribbean

AT

THE IMPERIAL COLLEGE OF TROPICAL AGRICULTURE

TRINIDAD, W.I.

A REPORT ON

CACAO RESEARCH

1957-1958

September, 1959

MYCOLOGICAL

The Regional Research Centre for the British territories in the Caribbean came into being on 1st September, 1955. The nucleus of the Centre has been formed from the staffs of the three research schemes for bananas, cocoa and soils which were wound up on 31st August.

The Cocoa Research Scheme made on 4th December, 1947, provided for the maintenance of type collections and for research on all problems connected with the cultivation, genetical improvement and health of the cocoa tree and of its fruit. In the agreed five-year research programme, effective from 1st September, 1956, it is stated that " Research on cacao has two main practical objectives : (*a*) the elaboration of techniques for removing factors that limit optimal yield and (*b*) evolution of high-yielding trees bearing beans of desirable flavour." It is recognised that the research of the Cocoa Section of the R.R.C. is not confined to Caribbean problems but, as far as local circumstances allow, is world-wide in its range.

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A REPORT ON CACAO RESEARCH

1957-1958

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Copies of this Report may be obtained from The Imperial College of Tropical Agriculture, Trinidad, W.I., or from the London Office, 40, Norfolk Street, W.C.2.

September, 1959 *Price: 15 shillings*

ST. AUGUSTINE, TRINIDAD, W.I.

THE REGIONAL RESEARCH CENTRE

of the British Caribbean

at

THE IMPERIAL COLLEGE OF TROPICAL AGRICULTURE

Incorporated by Royal Charter in 1926

G. A. C. HERKLOTS ... ... Principal and Director of Research.

Staff

of the

REGIONAL RESEARCH CENTRE

engaged on cacao research 1957-1958

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Introduction

HER Royal Highness the Princess Margaret paid a visit to the College on 24th April and together with Their Excellencies the Governor-General, Lord Hailes, and Lady Hailes and the Federal Prime Minister, Sir Grantley Adams and Lady Adams visited the cacao research plots. The Royal visit marked what proved to be the end of a long, exceptionally hot and dry spell. Because of the drought it had, therefore, been decided to water the guard row of trees of ICS 1 bordering the plot selected for demonstration and not to pick any pods for some weeks. In consequence the trees were laden with brightly coloured pods. A mat-shed was erected nearby and in this were arranged series of exhibits of pods, opened pods displaying the fresh beans, fermented beans, dried beans and samples of chocolate made in the laboratory nearby. There were also exhibits of shoots infected by Witches' broom and of pods showing the damage caused by Black-pod disease.

Messrs. Cope, Fennah and Murray were presented to H.R.H. the Princess Margaret and they then demonstrated the exhibits to her and to the other guests. Visitors from the neighbouring villages of Curepe, St. Augustine and Tunapuna watched the demonstration from seats in the nearby garage.

The first paper by F. W. Cope represents the results of ten years' study of the mechanism in the ovule of the cacao flower which determines whether the nucleus derived from the pollen grain from a flower of the same tree or of a tree of another clone can unite with the nucleus of the ovule to result in the formation of a normal seed—the bean of commerce. The work is a *magnum opus* and is likely to become a classic amongst research papers on cacao. It has been presented in greater detail in part fulfilment of the requirements of the University of London for a doctorate and has been rewritten as two papers which will be offered to the Editor of " Heredity." The paper here is a summary, with the minimum of technical genetic detail, and it is hoped will prove of interest to many. In the course of this work Cope estimates that he has examined some ninety thousand ovules under the microscope. His investigations have revealed " a type of incompatibility mechanism hitherto unknown in the plant kingdom."

An understanding of incompatibility is important to the cacao breeder. In some aspects, the incidence of incompatibility increases the difficulty of breeding improved cacao types ; in others, its incidence is positively helpful. Incompatibility mechanisms, as Cope points out, promote outbreeding which, by maintaining genetic variability in cacao populations, allows the breeder to select superior types for the benefit of the cocoa farmer and of the industry. Another beneficial aspect of incompatibility is the ease with which bulk seed of hybrid origin can be produced from self-incompatible parents. Cope concludes by stating, " If, in an area isolated from other cacao, a mixture of Amazon and Trinidad clones is planted, then all pods borne on the Amazon material will be cross pollinated with the Trinidad clone, since the former is self-incompatible. If the Trinidad parent is also self-incompatible, then all pods produced in the seed garden will arise from cross-pollination only."

The paper by R. G. Fennah may be the last in this series of reports from his pen as he resigned his post in August, 1958, to take up that of assistant director of the Commonwealth Institute of Entomology. His is a great loss to the R.R.C. for not only is he an entomologist with an international reputation—a world authority on some of the more difficult groups of Homoptera—but is a pioneer in the tropics in the field of insect ecology. His published papers exceed one hundred. He has served in the West Indies as entomologist since 1937, the last seven years of which he was on the staff of the Cocoa Research Scheme and of the Regional Research Centre. Fennah has lived up to the belief that a scientist trained in one discipline may make himself master of others by sheer hard work. His knowledge of the biochemistry and physiology of the cacao tree is considerable and in this paper, which is essentially an ecological study, the physiology of the tree and of the mealybugs are correlated. In the work described, the distribution of a population of mealybugs was followed for two years in plots where cacao trees were grown under different light intensities and were given different fertiliser treatments. The heaviest infestation was negatively correlated with light intensity, positively correlated with the application of nitrogen and negatively correlated with that of potassium, but showed no correlation with phosphorus. Heavy incidence of mealybugs was negatively correlated with vigour of the host plant, and this, in turn, was negatively correlated with depth of gravel below the soil surface. It is shown that these relationships can be accounted for by assuming that they are dependent on the effects of the different treatments on the trees in the plots.

The question of whether the stock influences the scion or the scion the stock on which it is grafted is one of importance. Experiments were initiated in 1951 to determine this and the results, as set out by D. B. Murray and F. W. Cope in their article, appear to be conclusive. Scion and stock do not interact : the combination of two comparable high-yielding clones does not result in plants yielding more beans than would have been obtained from each clone grown on its own roots. Only where a low-yielding weak criollo clone is concerned would it be possible to increase yield by grafting on to a more vigorous stock. The normal practice of propagating clones by cuttings on their own roots is strongly supported by the evidence presented.

Readers of this series of reports on cacao research need not be reminded of the classic researches carried out at River Estate since 1947. Most of the flat land at River Estate consists of fine and medium sands and sandy loams overlying coarse gravel ; internal drainage is free. This is

not typical of much of the land in which cacao is cultivated throughout the world. Moreover, the clones which do well in such light soils, e.g. ICS 1, do not necessarily do well in heavy soils. Clearly it is highly desirable that an area of heavy land with impeded drainage should be obtained not only to test the performance of clones grown in it, but also that knowledge might be obtained as to how best such land should be used for cacao cultivation. In the article by D. B. Murray he describes how the new sub-station at Las Hermanas was acquired and first steps in its development. Prior to development, the yield of dried cacao beans in the 1957-58 crop was a mere 130 lb. per acre.

The use of synthetic plastics is legion. R. Nichols describes how polythene bags may be used to facilitate the vegetative propagation of clonal cacao. He points out that the advantages are many. Each bag costs but 2½d. and may be used several times without deterioration and both labour and capital costs are negligible when compared with conventional methods. Transportation in bags is easy and the plants do not dry out even when carried for long distances.

The word gibberellic has crept into the English language in recent years along with sputnik. It was inevitable that the effect of gibberellic acid on seedlings and rooted cuttings of cacao had to be investigated and in his paper R. Nichols describes the results he obtained from his experiments. The effect of spraying well-rooted cuttings with a solution of fifty parts per million of GA was nil. The effect on seedlings was, however, considerable. Treated seedlings elongated much faster than the controls ; this is due to elongation of the internodes, with a corresponding increase in water content, but not with a parallel increase in dry weight. The young leaves are reduced in size and the growth is weak. Evidently treated seedlings cannot take in the extra nutrients required to match the increase in size. In plants sprayed at weekly intervals the terminal bud died, apical dominance in consequence was lost, the axillary buds sprouted and the seedling became bush-like.

In the previous report R. Nichols gave evidence that there are present in cacao tissue three active substances which he named cacao auxin I, cacao auxin II and an acid inhibitor. In his present paper he gives the results of an examination of these substances in the cherelles, or young fruits, which have presumably been made in or released from the ovules. The auxin activity is shown to be related to the wilting index of cherelles. An approximate inverse relationship is apparent between the wilt index and the auxin concentration : this is in accord with the view that wilt is controlled by the production of endogenous auxin. Evidence is presented that auxin I is similar to indoleacetic acid with the proviso that the transient nature of the colour reaction with $FeCl_3/HClO_4$ is not typical of IAA. Preliminary work on dormancy of buds of fan branches has yielded interesting results which encourage further research to determine what causes bud break and subsequent flushing.

Because the application of gibberellic acid to cacao seedlings produces " brooming " growth, following death of the terminal bud and the growth of lateral buds, it was decided to determine whether Witches' broom associated with infection by *Marasmius perniciosus* was in fact caused by the production of gibberellin-type substances by the fungus. W. F. Dudman and R. Nichols carried out experiments which prove that *M. perniciosus* does not produce a growth-stimulatory substance similar to that produced by *Fusarium moniliforme*.

Readers will recollect the papers by Paul Holliday in the last two reports on an inoculation technique for testing the resistance of seedlings to infection by *Marasmius perniciosus*. B. G. D. Bartley has made a detailed and critical comparison of the results obtained by inoculating in the laboratory seedlings of known crosses with plants in the field of the same type infected naturally. He shows that inoculations enable the most susceptible and the most resistant types to be readily distinguished, but that the resistance test is not sufficiently precise where the reaction is intermediate and variable. He states that the development of symptoms is usually complete at about three months from the inoculation date and that the final classification can be made then by an experienced person. Bartley concludes that, whilst experience has shown that the inoculation test is as suitable a method of obtaining the desired results as field tests alone, exposure of artificially inoculated plants to natural infection must be used as an accessory method of analysis of resistance.

The genus *Herrania*, indigenous to Central America and to northern South America, is very closely related to *Theobroma*. Whilst some species of *Herrania* occur in areas of South America where Witches' broom disease infects species of *Theobroma* there is only one recorded observation of *Herrania* being infected in the wild. In the collection of ten species of *Herrania* at River Estate vegetative and floral infection has been observed in three species. Two of these species occur in the Amazonian region where *Marasmius perniciosus* has not yet been found. This short paper by B. G. D. Bartley and E. F. Iton is illustrated by photographs of typical brooms on *Herrania*.

A wilt disease of cacao caused by *Ceratostomella fimbriata* is known from Costa Rica, Colombia, Venezuela and Ecuador. In March, 1958, several planters reported the rapid wilting and death of cacao trees in the Northern and Central ranges of Trinidad. *C. fimbriata*, in association with shot-hole borers, was shown to be the cause of the disease. This fungus is world-wide in its distribution and in 1936 was shown by Baker to be the cause of a minor pod rot of cacao, but it has never, locally, until now been known to cause disease of the vegetative parts of the cacao tree. In his paper E. F. Iton reviews the known distribution of the disease in Trinidad and describes its symptoms and the relationship of the disease to the siting of the borings made by the shot-hole borer. He then describes laboratory studies and the epidemiology of the disease ; the empirical control measures now in use at River Estate are outlined. He emphasises that more knowledge of the bionomics of the fungus and of the insects associated with the disease is required before a rational approach can be made to the problem of its control.

Fortunately, neither of the two strains of cacao virus present in Trinidad appears to be lethal. In this respect Trinidad is more fortunate than West Africa where the swollen shoot virus has been and still is a scourge. In Trinidad eradication of the virus has proved impracticable, but the disease is confined within a *cordon sanitaire*. River Estate lies within the infected area. Posnette and Thresh have both tried to inactivate virus in budwood by hot water treatment, but neither achieved success. D. B. Murray and J. T. Swarbrick have repeated their work with unfortunately similar results. They took the oppor-

tunity also to test how healthy budwood stood up to hot water treatment and found it more tolerant than diseased budwood. The lower thermal death point of healthy budwood was also investigated and it was found that at 30°C. (the lowest temperature possible with the equipment) forty minutes exposure did not result in loss of viability and that the time to bud break was considerably reduced by the treatment. This may have practical application.

The paper by D. B. Murray and L. A. Griffiths on the effects of delayed filling of cacao fermentation boxes is of considerable practical importance. The deterioration in the quality of Trinidad cocoa has been a source of complaint for some time. It would appear that the authors of this paper have satisfactorily demonstrated the major cause of this deterioration. Their results confirm the generally accepted impression that delay in completing the filling of the sweat box is a bad practice. The longer the period for filling the box the worse the result ; prolonging the period of fermentation will not correct the evil. If a cacao grower has only a small crop he must use smaller sweat boxes. The dimensions of a box should be not less than two or two-and-a-half feet each way ; such a box would hold between four hundred and eight hundred pounds of wet cacao.

The review by L. A. Griffiths, on the optimal conditions for micro-fermentation, will repay very careful study by all those who are concerned with fermentation whether in the laboratory or in the commercial sweat box. The author has paid particular attention to the techniques of Wadsworth and Howat and of Forsyth and Quesnel. He emphasises that the presence of acetic acid is essential and reviews the significance of aeration and of high temperatures and the necessity for maintenance of a high humidity in the fermenting vessel.

L. A. Griffiths, in his article on a laboratory procedure for micro-fermentation, takes the view that up till now no micro-fermentation methods have given or could give fermented beans of a quality comparable with those obtained by good commercial fermentation. He has himself developed a small-scale method the conditions of which approximate to those in a sweat box. Flavour assessment of beans so fermented has been satisfactory.

Four papers by G. K. Maliphant conclude this Report. The first deals with the nitrogen and phosphorus content of leaves taken from eight-year-old trees of ICS 1 in an experiment described in the previous Report. The effects of shade, no shade and of applications of fertilisers supplying nitrogen, potassium and phosphorus are listed.

In the second short paper the author refers to the fact, so far not completely understood, that after seven years for shaded and eight years for unshaded cacao, the significant correlation between girths at three-and-a-half years and successive crops no longer holds. The reason may be due to the elimination by then of weed competition.

In the third paper Maliphant refers to the different experiments at River Estate, to the fact that some have been abandoned and others are not yet yielding results requiring detailed treatment.

In the final paper the author gives tables showing the yields, over the crop years 1954 to 1958, of dry cacao per acre from experiments involving different combinations of shade and spacing with and without fertilisers. Responses to nitrogen were higher in the absence of shade and highest when given twice a year, May and September, along with phosphate. Responses to phosphate likewise were higher in the absence of shade. On the other hand the response to potash was higher in the presence of shade.

Conferences

The Conference of The Cocoa, Chocolate and Confectionery Alliance, was held in London from 10th to 12th September, 1957, and was attended by the principal, R. G. Fennah, F. W. Cope, A. L. Jolly and B. G. D. Bartley. The following papers were presented :

" Trinitario-Scavina hybrids—New prospects for cocoa improvement " (B. G. D. Bartley) Conference Report pp. 36 to 40.

" The cocoa tree in relation to its environment " (R. G. Fennah and D. B. Murray) Conference Report pp. 222 to 227.

The Seventh Meeting of the Inter-American Technical Committee on Cacao was held at Palmira, Colombia, from 13th to 19th July, 1958. The meeting was attended by B. G. D. Bartley and G. K. Maliphant and the following papers were presented :

" High yields in cacao field experiments and their significance in future cacao research " (G. K. Maliphant).

" Observations on the reaction of young cacao seedlings to artificial infection by *Marasmius perniciosus*, Stahel " (B. G. D. Bartley).

A report on the meeting was submitted by B. G. D. Bartley.

Publications

Papers by members of the cocoa research staff of the R.R.C., which have appeared in journals other than the reports of the Conferences mentioned above and since the Report for the years 1955-1956, which refer directly or indirectly to cocoa research.

Griffiths, L. A. (1957) : Separation and Identification of Aromatic Acids in Plant Tissues by Paper Chromatography. *Nature, Lond.*, **180**, 286-287.

Griffiths, L. A. (1957) : Detection of the Substrate of Enzymic Browning in Cacao by a Post-chromatographic Enzymatic Technique. *Nature, Lond.*, **180**, 1373-1374.

Griffiths, L. A. (1958) : Occurrence of Gentisic Acid in Plant Tissues. *Nature, Lond.*, **182**, 733-734.

Griffiths, L. A. (1958) : Phenolic Acids and Flavonoids of Theobroma Cacao L. Separation and Identification by Paper Chromatography. *Biochem. J.*, **70**, 120-125.

Jones, T. A. and Maliphant, G. K. (1958) : High Yields in Cacao Field Experiments and their Significance in Future Cacao Research. *Trop. Agriculture, Trin.*, **35**, 272-275.

Murray, D. B. (1957) : The Role of Shade and Fertilisers in Cocoa Cultivation. *J. Agric. Soc. Trin. Tob.*, **57**, 193-207.

Nichols, R. (1958) : Propagation of Cacao in Plastic Bags. *Nature, Lond.*, **181**, 580.

Nichols, R. (1958) : A Possible Source of Error in the Chemical Detection of Indolyl Acetic Acid in Plants. *Nature, Lond.*, **181**, 919-920.

Incompatibility in *Theobroma cacao**

by

F. W. Cope

Introduction

TEN years of investigations on incompatibility in cacao have revealed a type of incompatibility mechanism hitherto unknown in the plant kingdom. The failure of an incompatible pollination to set fruit results from the presence, in the pollinated ovary, of a proportion of ovules in which fusions between the male gametes and their respective female counterparts have not taken place (" non-fusion " ovules). The ovarian content of such ovules is regularly either 25%, 50% or very nearly, if not actually 100% of all ovules fertilised ; the other ovules in the ovary show normal fusion between one male gamete and the egg nucleus and between the second male gamete and the polar nuclei. Three types of self-incompatible tree can, therefore, be recognised : (1) those which give a 1 : 3 ratio for non-fusion to normal ovules on selfing ; (2) those which give a 1 : 1 ration on selfing ; (3) those which give 1 : 0 on selfing. The regularity shown in the incidence of non-fusion ovules strongly suggests that the phenomenon of non-fusion is under genetic control.

Compatible pollinations, on the other hand, are characterised by the lack of non-fusion ovules in the pollinated ovary. Ovules wherein fusions have not taken place are very sporadically encountered in compatibly pollinated ovaries, but the very low incidence (one such ovule in every 440 normal ovules) and extremely irregular occurrence of such ovules suggest that their appearance has no profound significance.

In the vast majority of reported cases of incompatibility in the flowering plants a reaction of the pollen tube with the style is involved. In incompatible pollinations the germination of the pollen, when applied to the stigma, may be totally prevented ; or if germination does take place, then pollen tubes carrying male gametes which are incompatible with the female plant are arrested in their growth and so fail to deliver the gametes to the ovules. The reaction of the pollen towards the diploid female tissues may in some cases (e.g. *Nicotiana*) be determined by the haploid genotype of the individual pollen grains ; in such circumstances, the incompatibility reaction is said to be under gametophytic control. In other instances, however, (e.g. *Primula*) the pollen's reaction is determined by the diploid genotype of the plant which produces the pollen ; though the pollen grains may differ in genotype, they all behave phenotypically similarly towards the style ; such cases are said to exhibit sporophytic control of the incompatibility reaction. The genetic control of the incompatibility reaction may be exercised by one or more loci ; where a single locus is involved, as in *Nicotiana*, that locus may be occupied by any of a large series of alternative alleles (a multiple allelomorphic series of S-alleles) ; where more than one locus controls the reaction (as in *Primula*), only two alternative alleles at the S locus exist and the whole system of control involves the interaction of other loci with the S locus. Where sporophytic control of the reaction of the pollen to the style exists, the possibility always arises that the S-alleles (if belonging to a multiple allelomorphic series), may show dominance relationships.

In cacao, the incompatibility reaction does not involve the style. Incompatible pollen tubes grow down the style just as readily and as rapidly as compatible tubes and deliver their gametes into the embryo-sacs where fusion with egg and polar nuclei may, or may not, take place. Knight and Rogers (1953, 1955) demonstrated that the control of the cacao incompatibility mechanism was sporophytic ; they suggested that a single locus was concerned which showed multiple allelomorphism, with dominance and equality relationships between the alleles. The investigations briefly reported in this paper support the general theory of Knight and Rogers, but their simple hypothesis is found to need modification to accommodate newly discovered facts. In particular, two other loci are required, besides the S locus ; these have been called A and B and are thought to be concerned with the production of a non-specific " incompatibility precursor," upon which the S-alleles act to give highly specific incompatibility reactions.

Materials and Methods

The cacao materials used in the investigations are of diverse origins. All Trinitario trees studied belong to the ICS series of clones. Amazon materials stem from Pound's (1938) collection. Three Ecuadorian clones were examined. Numerous seedling progenies, raised under the plant breeding programme, were investigated. Some of the trees on which Knight and Rogers based their conclusions were included in the studies.

The investigations concerned themselves with the incidence of non-fusion and normal ovules in ovaries taken from flowers guarded from insect visits and carefully hand-pollinated. The cacao ovaries were removed from the flower at 72 hours after pollination and placed for 24 hours in " Craf " (Randolph, 1935) fixative and taken up to paraffin wax through a series of ethyl-tertiary butyl alcohol mixtures (Johansen, 1940) and cut into serial microtomed sections of 15μ thickness. Haidenhain's iron-haematoxylin was used to stain the sections, differentiation being effected with a saturated solution of picric acid. The slides were then examined under a magnification of 900 times, each loculus being followed through until all the fertilised ovules in the ovary had been examined and assessed as normal or non-fusion.

*This paper is a condensation of two papers which, it is hoped, will be accepted for publication in " Heredity."

TABLE I

Percentage of ovules showing non-fusion, in relation to time after pollination, for selfings on ICS 43 and ICS 53

		Hours from pollination			
		24	48	72	96
Unfused ♂ with egg	ICS 43	39.7	29.7	24.1	23.9
	ICS 53	37.8	23.2	22.2	26.3
Unfused ♂ with polars	ICS 43	32.7	28.8	20.7	19.1
	ICS 53	31.3	20.0	19.8	26.3

Results

Early studies on self-pollinated flowers of ICS 43 and ICS 53, both known from previous hand-pollinations to be self-incompatible, revealed that at 72 hours from pollination the ovaries contained approximately 25% of non-fusion ovules. Examination of a series of ovaries fixed at 24, 48, 72 and 96 hours after pollination showed that gametic fusion, where it took place, was achieved very largely in the first 48 hours after pollination; so that with ovaries fixed at 72 hours the investigator can be reasonably certain that whatever non-fusion ovules are seen would not be reduced in number by leaving the flower on the tree for a longer time (Table I). The non-fusion ovules contained a male gamete lying in contact with the egg; the second male gamete was usually lying, similarly, in contact with the paired polar nuclei (Figs. 1 and 2), but embryo-sacs were occasionally seen wherein the male nucleus lay separated from the polars. If the second male gamete does not make contact with the polar nuclei, the latter often become separated and may even divide to give four haploid nuclei. Very rarely the male gamete divides simultaneously with the polars, so that six haploid nuclei, external to the egg-apparatus, are found in the embryo-sac. Non-fusion ovules can often readily be discerned under low-power magnification, for the intensity of staining is low in the integuments and nucellus, except that the nucellar cells immediately adjacent to the embryo-sac frequently stain intensely and show signs of cellular breakdown. The normal ovules (approximately 75% in content) present in the ovary contain a somewhat enlarged zygote, which may be uni- or binucleolate, and a triploid primary endosperm body which, at 72 hours after pollination, has often divided to give two or four endosperm nuclei. Mitotic activity in the integuments and nucellus of normal ovules in incompatibly pollinated ovaries is not maintained, however, at the same level as in normal ovules in compatibly pollinated ovaries. Activity in non-fusion ovules reaches a peak at 24 hours from pollination and then rapidly subsides to the point of extinction.

With flowers of ICS 53 which have been compatibly pollinated (with ICS 1) non-fusion ovules are entirely lacking in ovaries fixed at 72 hours after pollination.

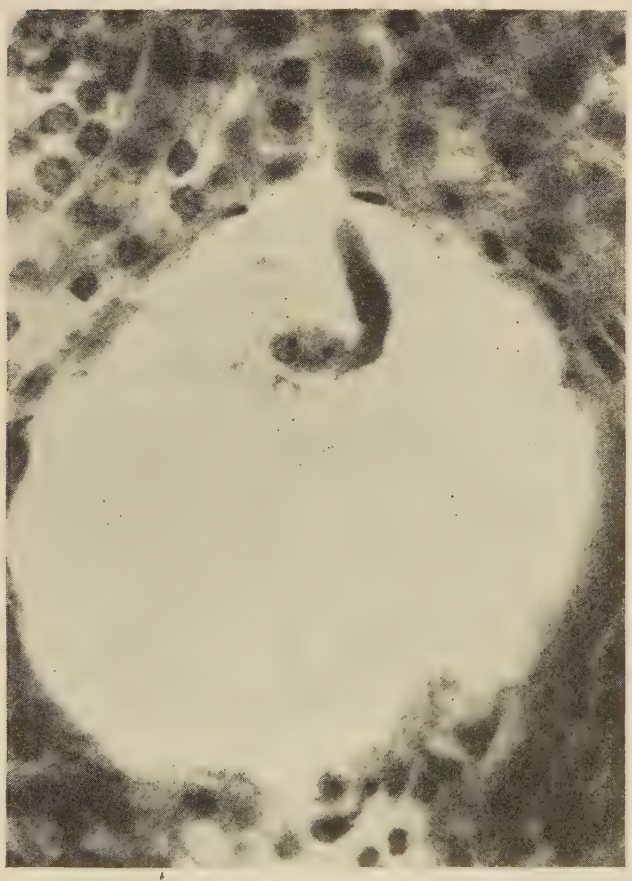

FIGURE 1

Cross-section of a "non-fusion" cacao ovule, 72 hours after incompatible pollination. The small male gamete lies in contact with the larger egg nucleus. ×925.

FIGURE 2

Cross-section of a "non-fusion" cacao ovule, 72 hours after incompatible pollination. The second male gamete lies in contact with the two polar nuclei. ×925.

TABLE II

Percentage of ovules showing non-fusion, in relation to time after pollination, for compatible crossings on ICS 43 and ICS 53

		Hours from pollination			
		24	48	72	96
Unfused ♂ with egg	ICS 43	32.4	5.0	1.7	1.4
	ICS 53	17.5	7.6	1.5	0.0
Unfused ♂ with polars	ICS 43	19.8	2.9	1.7	0.5
	ICS 53	5.3	3.3	2.5	0.0

With ICS 43, compatibly pollinated, non-fusion ovules very occasionally are seen (Table II). Normal ovules in compatibly pollinated ovaries of ICS 43 and ICS 53 can be distinguished from normal ovules in the incompatible selfings only by the fact that mitotic activity in nucellus and integuments of the latter is much lower than that of the former.

Because selfed ovaries of ICS 43 and ICS 53 both contained approximately the same 25% content of non-fusion ovules at 72 hours from pollination, the investigations were widened in scope to embrace 50 self-incompatible ICS clones in order to see whether the 25% non-fusion ovule content was something fundamental to the incompatibility mechanism. It did indeed prove to be so, for in 150 selfed ovaries (triplicates of the 50 clones) totals of 1,434 non-fusion and 4,275 normal ovules were counted (Table III). The non-fusion ovule content averaged, therefore, 25.12% in the 150 ovaries. Using the weighted half-logit transformation on the individual ovary counts, it was demonstrated that the within-tree variance of the ratio of non-fusion ovules to normal ovules did not differ significantly

TABLE III

Ovule counts in triplicate from selfed ovaries of 50 self-incompatible ICS clones. L.H. number refers to ovules showing non-fusion of gametes, R.H. to ovules showing normal fusion.

ICS			ICS			ICS			ICS			ICS		
	5	19		8	32		9	34		12	28		11	24
2	5	17	19	11	34	43	8	28	58	11	27	80	9	33
	7	23		9	38		10	24		16	25		9	34
	11	27		9	18		6	36		14	31		10	35
3	11	31	22	3	16	46	11	34	60	12	25	82	12	34
	8	28		11	19		11	33		10	32		11	35
	6	24		9	30		8	32		6	21		17	25
4	14	25	26	7	32	47	10	36	64	12	31	83	7	30
	7	28		14	25		9	36		9	28		10	31
	11	23		10	32		9	26		10	30		15	23
5	10	29	34	12	26	48	12	25	66	12	25	84	10	30
	9	32		11	38		11	32		6	30		10	31
	9	24		9	28		8	32		9	26		7	28
11	14	24	35	8	30	50	5	28	71	10	24	86	7	32
	4	27		13	32		6	31		9	30		9	22
	9	30		14	26		12	27		7	25		8	32
13	2	22	37	9	33	51	11	29	72	6	27	87	7	36
	12	36		15	26		13	27		10	26		12	30
	14	23		7	28		11	28		5	20		9	27
15	6	34	39	11	27	52	12	28	74	11	24	89	4	29
	8	24		10	35		12	30		10	34		3	35
	10	25		12	24		7	23		8	31		12	24
16	10	32	40	11	35	53	7	27	75	10	37	92	5	32
	12	36		9	32		4	35		12	29		11	37
	7	24		9	30		11	21		11	20		10	25
17	9	32	41	6	31	56	10	26	77	6	32	94	8	30
	14	20		12	26		13	27		10	28		9	28
	13	30		12	27		10	38		8	25		10	24
18	11	31	42	11	27	57	16	34	78	5	18	99	3	22
	12	33		12	28		7	30		9	26		10	17

Totals : 1,434 4,275

Average number of fertilised ovules per ovary 38.1

TABLE IV

Frequency distribution of contingency-χ^2 values (calculated from ratio, 1,434 : 4,275) for the 150 ovule counts of Table III.

x^2 range	Actual frequency	Expected frequency	x^2
0.0000-0.0039	8	7.5	0.033
0.0039-0.0158	12	7.5	2.700
0.0158-0.0642	12	15.0	0.600
0.0642-0.148	13	15.0	0.267
0.148 -0.455	38	30.0	2.133
0.455 -1.074	33	30.0	0.300
1.074 -1.642	9	15.0	2.400
1.642 -2.706	14	15.0	0.067
2.706 -3.841	5	7.5	0.833
Over 3.841	6	7.5	0.300
Totals	150	150.0	9.633 (9 df) $p=0.4$

from the between-tree variance. The frequency distribution, by magnitude, of the 150 contingency-χ^2 values, calculated from the overall ratio, 1,434 : 4,275, showed a good fit with expectation (Table IV), so that though the ratio of non-fusion to normal ovules does vary from ovary to ovary, the " spread " in the ratio is neither larger nor smaller than the theory of random sampling would require. The two statistical analyses strongly suggest that the appearance of non-fusion ovules in the 150 selfed ovaries is under the control of some simple factor which, random-sampling effects apart, is remarkably constant in effect. Analysis was also made of the frequency of encounter of from zero to n non-fusion ovules (where n is the number of fertilised ovules) in groups defined by the loculus in the 150 samples ; once again, the frequency distribution showed good fit with expectations (Table V) based on the binomial expansion of $(0.2512+0.7488)^n$. The deduction may be made from this analysis that the encounter between gametes unable to fuse in the embryo-sac is a matter controlled by strictly random processes ; and that what happens between gametes in one ovule is quite independent of events in other ovules.

Thirty-one self-compatible ICS clones were investigated in their reaction towards self-pollination. A total of 2,052 fertilised ovules was examined and only one of these was of the non-fusion type.

Proof was made that all pollen grains (and not a recognisable fraction only) in incompatible matings are capable of delivering gametes into the embryo-sac. Counted numbers of pollen grains were applied to incompatible stigmas, and the ovaries sectioned. In all cases the number of ovules fertilised was only very little less than the number of grains applied.

At this stage of the investigation it seemed more than reasonably certain that the presence of about 25% non-fusion ovules in ovaries was correlated with the abscission of the selfed flower, and that the absence of these ovules led to the flower's retention and development into a fruit. Still further proof was sought, however, in repeating the work of Posnette (1945) on a group of self-incompatible Amazon clones. Posnette had shown that self-pollinations on 11 clones of Nanay (NA), Parinari (PA) and Iquitos (IMC) origins always resulted in the total abscission of the flowers. Certain cross-pollinations, however, within and between the NA, PA and IMC materials led to fruit setting. It was gratifying to find that in every case for which Posnette reported a failure to set fruit, non-fusion ovules appeared in the ovaries ; and that in every case of cross-compatibility reported, non-fusion ovules were lacking. In the incompatible matings, 629 non-fusion ovules (24.3%) appeared amongst 2,594 ovules examined. In the compatible crosses not a single non-fusion ovule was encountered in 5,140 fertilised ovules.

The range of clones investigated in this section of the work also included three Ecuadorian clones, E 5, E 15 and E 17, and two Amazon clones, SCA 6 and SCA 12. At the outset, the Ecuadorian material was assumed to be self-incompatible, but sections of ovaries derived from selfings of E 15 and E 17 showed that non-fusion ovules were lacking ; the apparent self-compatible status for these two clones was fully established when selfings done on them resulted in good fruit setting. The clone, E 5, on the other hand, yielded non-fusion ovules on selfing and repeated hand-pollinations failed to set any pods ; this clone proved also to be cross-incompatible with two of the NA clones investigated. Four selfed ovaries of SCA 6 gave counts :—

Non-fusion Ovules	*Normal Ovules*
25	26
14	26
22	19
18	13
79	84

so that it became apparent that SCA 6 differed from all other self-incompatible material so far examined in giving approximately 50% non-fusion and 50% normal ovules when self-pollinated.

Statistical analyses of all results obtained from a repetition and extension of Posnette's work (excluding SCA 6) showed that : (*a*) the within-tree variance of the ratio of non-fusion to normal ovules in incompatibly pollinated ovaries was of much the same magnitude as the between-tree variance, irrespective of whether the incompatible pollination was a selfing or a cross : (*b*) the actual frequency distribution of contingency-χ^2 values fitted well with expectation : (*c*) the encounter of gametes unable to fuse was a random process.

The cross-relationships of fourteen self-incompatible ICS clones and two self-incompatible Amazon clones were studied. All combinations of ICS parents gave the now familiar incidence of about 25% non-fusion ovules in the ovary, showing that besides being self-incompatible, the clones were mutually cross-incompatible. 1,801 non-fusion ovules (25.3%) appeared in a total of 7,122 fertilised ovules examined (Table VI). The two Amazon clones, PA 7 and NA 32, showed themselves to be cross-compatible with all 14 of the ICS clones ; no non-fusion ovules were detected in 1,402 examined. These results confirm what had previously been experienced in the plant-breeding programme, *viz.*, that no self-incompatible ICS clones have yet been found that are cross-compatible, yet Trinidad materials appear always to be cross-compatible with cacao material from the Amazon basin.

Table V

Frequency distribution of numbers of ovules (r) showing non-fusion in loculi containing 5-10 fertilised ovules (n). Expected frequencies are based on expansion of $(0.2512 + 0.7488)^n$. Data for 150 counts of Table III.

r	Number of fertilised ovules in loculus (n)											
	5		6		7		8		9		10	
	Act.	Expd.	Act.	Expd.	Act.	Expd.	Act.	Expd.	Act.	Expd.	Act.	Expd.
0	11	8.71	12	12.16	24	22.70	19	22.83	11	9.62	3	3.88
1	12	14.61	24	24.48	46	53.32	79	61.28	27	29.05	18	13.02
2	10	9.80	19	20.53	44	53.65	61	71.95	36	38.98	24	19.65
3	4	3.29	9	9.18	38	30.00	49	48.27	37	30.51	11	17.57
4	0	0.55	4	2.31	17	10.06	17	20.24	16	15.35	13	10.32
5	0	0.04	1	0.31	3	2.03	5	5.43	3	5.15	1	4.15
6			0	0.02	0	0.23	1	0.91	0	1.15	2	1.16
7					0	0.01	0	0.09	0	0.17	0	0.22
8							0	—	0	0.01	0	0.03
9									0	—	0	—
10											0	—
χ^2	1.076		2.247		9.913		8.024		3.847		7.139	
p	0.8		0.7		0.05-0.10		0.15		0.6		0.2	

Table VI

Ovule counts from cross-pollinations between 14 self-incompatible Trinidad (ICS) clones and two Upper Amazon clones, in duplicate. L.H. number refers to non-fusion ovules, R.H. to normal ovules.

	ICS 3	ICS 11	ICS 15	ICS 26	ICS 34	ICS 39	ICS 43	ICS 46	ICS 50	ICS 52	ICS 60	ICS 74	ICS 87	ICS 89	PA 7
ICS 11	10 23 8 27														
ICS 15	10 22 9 24	14 23 9 31													
ICS 26	7 13 13 28	6 24 8 31	6 19 13 19												
ICS 34	12 33 10 34	12 35 12 25	9 34 6 20	14 28 8 30											
ICS 39	9 33 15 31	10 34 6 41	6 31 5 17	6 36 8 32	12 31 10 30										
ICS 43	8 35 11 39	11 29 8 25	10 36 8 33	10 34 8 38	16 32 10 35	9 36 14 32									
ICS 46	9 30 13 30	9 32 10 32	8 34 8 25	14 24 6 23	7 34 7 37	10 28 11 36	15 27 9 32								
ICS 50	5 34 11 37	3 33 6 31	7 21 8 29	6 33 15 22	14 29 11 26	4 32 5 27	10 28 11 21	6 31 13 24							
ICS 52	8 34 8 33	13 36 12 31	9 22 9 28	11 28 3 24	10 30 15 28	13 25 10 24	12 25 3 31	15 31 8 36	5 28 9 38						
ICS 60	11 34 10 33	15 31 10 38	12 35 8 32	11 31 12 30	8 34 9 34	4 20 13 28	13 23 12 25	10 23 9 29	13 28 11 28	12 25 10 29					
ICS 74	16 30 13 29	10 37 13 34	13 32 9 26	6 37 13 34	10 31 13 35	10 34 12 30	8 33 16 31	14 28 12 26	11 27 6 28	13 24 18 29	8 34 11 33				
ICS 87	12 30 12 27	12 32 6 37	10 35 14 28	12 31 10 29	11 33 12 27	8 31 9 35	9 36 8 35	7 31 13 29	10 29 10 25	6 24 8 26	10 29 7 23	6 37 11 34			
ICS 89	10 28 8 24	8 22 9 24	10 23 6 31	10 27 8 27	9 23 9 25	8 22 9 27	9 30 13 23	11 25 11 27	9 27 13 25	10 25 10 26	13 20 9 25	4 32 12 22	9 26 13 23		
PA 7	0 43	0 40	0 44	0 46	0 41	0 34	0 42	0 45	0 44	0 45	0 32	0 46	0 36	0 45	
NA 32	0 59	0 55	0 53	0 60	0 55	0 45	0 57	0 55	0 55	0 55	0 58	0 46	0 60	0 56	0 40

Totals (from ICS combinations): 1,801 5,321

Average number of fertilised ovules per ovary: 40.3

In the course of assessing the 100 ICS clones for self-compatibility/incompatibility by examination of the selfed ovary, one self-incompatible clone, ICS 90, gave counts suggesting that the incidence of non-fusion ovules was nearly 100% in selfed ovaries. Seven selfings gave counts :—

Non-fusion Ovules	*Normal Ovules*
27	0
29	1
25	2
32	1
25	2
39	1
40	1
217	8

Here, then, for the first time was an example of yet a third type of self-incompatible tree which, on selfing, gives only very few normal ovules (and occasionally none).

In 1956 the author was privileged to visit the West African Cocoa Research Institute in Ghana and to be able to make self- and cross-pollinations on some of the cacao genotypes used by Knight and Rogers. The counts obtained from sectioned ovaries are given in Table VII. According to Rogers, the S-alleles in the population studied bear to one another the relationship, $S_0 = S_1 > S_2 = S_3 > S_4 > S_5$, this dominance and equality series having been established by breeding experiments. Non-fusion ovules appeared in all ovaries from selfings or crosses reported by Knight and Rogers as incompatible (as judged by the abscission of the flowers) : only normal ovules were found in ovaries from cross-pollinations which these authors reported as compatible, leading to fruit setting. Selfings on genotypes which combine alleles of equal

TABLE VII

Ovule counts from self- and cross-pollinations on some genotypes investigated by Knight and Rogers. L.H. number refers to non-fusion ovules, R.H. to normal ovules.

♀ \ ♂	$S_{1\cdot2}$ $S_{1\cdot4}$	$S_{2\cdot3}$	$S_{2\cdot5}$	$S_{3\cdot4}$	"$S_{5\cdot5}$"
$S_{1\cdot2}$ $S_{1\cdot4}$	10 37 11 33 11 38 13 35 13 34 11 40 11 39	0 43 0 39	0 47 0 37	0 49 0 29	0 45
$S_{2\cdot3}$	0 41 0 52	22 24 24 19 24 26 24 25 22 30 24 23 23 28 25 19	8 36 6 17 10 37 10 41	9 31 9 35 12 32	0 47 0 47
$S_{2\cdot5}$	0 43 0 46	11 33 19 31 11 44	14 29 12 35 7 34 5 24 5 21 12 38	0 48 0 48	0 54 0 49
$S_{3\cdot4}$	0 49 0 49	15 32	0 53 0 39	7 30 14 34 13 34 15 33 8 41 14 34	0 45 0 52
"$S_{5\cdot5}$"	0 35 0 37	0 44 0 40	0 40 0 47	0 47 0 47	14 28 10 30 15 26 12 34 14 29 11 31
♀ \ ♂	$S_{0\cdot1}$	$S_{1\cdot2}$	$S_{2\cdot5}$		
$S_{0\cdot1}$	22 30 29 22 29 24 27 26	11 39	0 53 0 50		

$S_0 = S_1 > S_2 = S_3 > S_4 > S_5$

dominance ($S_{0\cdot1}$ and $S_{2\cdot3}$) gave counts of 295 non-fusion and 296 normal ovules ; the counts from the 12 individual ovaries are statistically homogeneous. Selfings on genotypes which combine an allele dominant to the other ($S_{1\cdot2}$, $S_{2\cdot5}$, $S_{3\cdot4}$) all gave a 25% content of non-fusion ovules in the ovary ; 206 non-fusion ovules (24.3%) and 643 normal ovules were counted. The selfing of the homozygous $S_{5\cdot5}$ genotype of Knight and Rogers did not, however, give the anticipated approach to a 100% content of non-fusion ovules ; the counts obtained suggest that this material falls into the 25% non-fusion class of self-incompatible tree. Rogers (private communication) has agreed that the $S_{5\cdot5}$ genotype might just as well be $S_{5\cdot6}$ (where $S_5 > S_6$) : this assumption does not in any way invalidate the hypothesis put forward by Knight and Rogers, and merely implies that their three original parent clones have between them six, and not five, different S alleles. Cross-pollinations between one genotype with equal alleles and another with one of these equal alleles dominant to its partner ($S_{0\cdot1} \times S_{1\cdot2}$, $S_{2\cdot3} \times S_{3\cdot4}$, $S_{2\cdot3} \times S_{2\cdot5}$) all gave a 25% incidence of non-fusion ovules ; 131 non-fusion ovules (24.3%) and 408 normal ovules were counted in these crosses. All other combinations of parental genotypes, i.e. crosses between genotypes which do not have a common dominant allele ($S_{1\cdot2} \times S_{3\cdot4}$, $S_{1\cdot2} \times S_{2\cdot5}$) or between a genotype with equal alleles and another where neither of the equal alleles is the dominant ($S_{2\cdot3} \times S_{1\cdot2}$), gave only normal ovules in the cross-pollinated ovaries. Table VII clearly shows the similarity of the results from reciprocal pollinations.

Fourteen groups of progenies, arising from selfings on and crosses between parents of known compatibility status, were investigated. The parents were :—

ICS 1, self-compatible (SC).

ICS 6, do.

ICS 44, do.

ICS 45, do.

ICS 60, self-incompatible, giving 25% non-fusion ovules in selfed ovaries (25% SI).

ICS 16, do.

SCA 12, do.

SCA 6, self-incompatible, giving 50% non-fusion ovules in selfed ovaries (50% SI).

Results obtained from the progenies, in respect of self-pollination, sib-pollination and back-pollination to both parents, are given below.

(1) ICS 1 selfed — All progeny SC.

(2) ICS 1 × ICS 6 — do.

(3) ICS 1 × ICS 44 — do.

(4) ICS 45 selfed — do.

(5) ICS 1 × ICS 45 ; (6) ICS 45 × ICS 1 — All progeny 25% SI and cross-incompatible (25% non-fusion ovules). All progeny cross-compatible with both parents.

(7) ICS 1 × ICS 16 ; (8) ICS 60 × ICS 1 — Approximately equal numbers of SC and 25% SI progeny. All progeny cross-compatible with SC parent. 25% SI progeny cross-incompatible with SI parent (25% non-fusion ovules).

(9) ICS 60 × ICS 45 ; (10) ICS 45 × ICS 60 — (*a*) 21 100% SI progeny. (*b*) 28 25% SI progeny. (*c*) 10 SC progeny. 3 : 3 : 2
All progeny cross-compatible with ICS 45.
Group (*a*) gives 50% non-fusion ovules with ICS 60.
Group (*b*) gives 25% non-fusion ovules with ICS 60.
Group (*c*) cross-compatible with ICS 60.

(11) ICS 1 × SCA 12 — All progeny 25% SI, falling into two approximately equal groups, (*a*) and (*b*).
All progeny cross-compatible with ICS 1.
Groups (*a*) and (*b*) are inter-compatible, intra-incompatible (25% non-fusion ovules).
Group (*a*) cross-incompatible with SCA 12 (25% non-fusion ovules).
Group (*b*) cross-compatible with SCA 12.

(12) ICS 1 × SCA 6 — All progeny 25% SI, falling into two approximately equal groups, (*a*) and (*b*).
All progeny cross-compatible with ICS 1.
All progeny cross-incompatible with SCA 6 (25% non-fusion ovules).
Groups (*a*) and (*b*) are inter-compatible, intra-incompatible (25% non-fusion ovules).
Group (*a*) is cross-incompatible with group (*a*) of previous cross (25% non-fusion ovules).

(13) ICS 60 × SCA 12 — (*a*) One quarter progeny 50% SI.
(*b*) Three-quarters progeny 25% SI.
Group (*a*) cross-incompatible with both parents (25% non-fusion ovules).
Group (*b*) gives three sub-groups (i), (ii) and (iii).
(i) cross-compatible with ICS 60, cross-incompatible with SCA 12 (25% non-fusion ovules).
(ii) cross-compatible with SCA 12, cross-incompatible with ICS 60 (25% non-fusion ovules).
(iii) cross-compatible with both parents.

(14) ICS 60 × SCA 6 — (*a*) Half progeny 50% SI.
(*b*) Half progeny 25% SI.
Group (*a*) cross-incompatible with both parents (25% non-fusion ovules).
Group (*b*) cross-compatible with ICS 60, cross-incompatible with SCA 6 (25% non-fusion ovules).

In the course of examining very many ovules, some observations were made to the effect that in the absence of pollen-tube entry into the embryo-sac, the egg nucleus occasionally fuses with one of the synergid nuclei. Some such cases were seen in early studies on ICS 43 and ICS 53, where guarded flowers were left unpollinated and then sectioned. The phenomenon is of interest in suggesting that female nuclei, of identical genetic constitution, experience no barrier to fusion, such as appears to be the case between male and female gametes in certain circumstances ; haploid male gametes are, therefore, in a condition physiologically different from that of haploid female nuclei.

Genetic interpretation

The results obtained from materials studied by Knight and Rogers very clearly point to the fact that in incompatible matings the gametes which do not fuse are those

which carry the same dominant allele. $S_{2\cdot3}$ and $S_{2\cdot5}$ are cross-incompatible, 25% non-fusion ovules appearing in the cross-pollinated ovary whichever way the cross is made; these two genotypes have S_2 in common. In the crosses, $S_{1\cdot2}\times S_{2\cdot3}$ and $S_{1\cdot2}\times S_{2\cdot5}$, non-fusion ovules are absent, so that S_2 gametes from $S_{1\cdot2}$ can readily fuse with S_2 gametes from $S_{2\cdot3}$ and $S_{2\cdot5}$. In $S_{1\cdot2}$ the S_2 gametes carry the recessive allele, whilst in $S_{2\cdot3}$ the S_2 allele is co-dominant with S_3 and in $S_{2\cdot5}$ it is dominant to S_5. The situation is, therefore, that the reaction of S_2 gametes towards one another depends upon the diploid constitution of the two parents producing the S_2 gametes. If both parents have S_2 as a dominant, the cross fails and 25% non-fusion ovules appear; if one parent has S_2 as a dominant and the other has S_2 as a recessive, the cross succeeds and non-fusion ovules are absent; where S_2 is recessive in both parents, the cross succeeds, provided that the alleles dominant to S_2 are not identical.

If $S_{2\cdot3}$ and $S_{2\cdot5}$ are cross-incompatible because of the common dominant, then it is a perfectly reasonable assumption to make that the 25% incidence of non-fusion ovules in the selfing of $S_{2\cdot5}$ is occasioned by S_2 male gametes meeting S_2 egg nuclei. When $S_{2\cdot3}$ is selfed, 50% non-fusion ovules appear in the ovary; half of these non-fusions are ascribable to encounter between S_2 gametes, so that the other 25% must be the result of encounter between S_3 gametes. In $S_{2\cdot3}$, the two alleles are of equal dominance, and so all gametes are "activated" against their own kind—but not against one another.

It is unfortunate that no self-incompatible cacao tree has yet clearly been identified by its breeding behaviour as being homozygous for an S-allele, but the conclusion seems inescapable that the 100% non-fusion self-incompatible types must be homozygous; when crossed with a genotype carrying the same allele as a dominant, 50% non-fusion ovules would be expected ($S_{2\cdot2}\times S_{2\cdot3}$). In incompatible crosses, as with selfings, the non-fusion ovule content is always 25%, 50% or 100%.

The fact that S_2 gametes from $S_{1\cdot2}$ can fuse with S_2 gametes from $S_{2\cdot5}$, but S_2 gametes from $S_{2\cdot4}$ cannot fuse with S_2 gametes from $S_{2\cdot5}$, indicates sporophytic control of the incompatibility reaction. The actual allele carried by a gamete does not of itself determine its fusion or non-fusion behaviour with other gametes of the same allelic constitution; the dominance relationships between the parental alleles determines whether or not two gametes will fuse.

If sporophytic control is, therefore, the rule in cacao, how is it that all gametes from any given genotype are not physiologically identical in properties? Why does the selfing of $S_{1\cdot2}$, say, give only 25% non-fusion ovules and not 100%? The answer must lie in the supposition that the S locus has a two-step action, one before meiosis and another after meiosis. The pre-meiotic action of the S locus establishes the sporophytic control of the incompatibility system, whilst the post-meiotic action is required to ensure that all gametes (except in homozygous genotypes) are not identical in their fusion behaviour.

Turning now to self-compatible genotypes, the evidence provided by studies of progenies strongly suggests that self-compatible trees breed true to the compatible conditions when selfed; this in turn suggests that self-compatible genotypes are homozygous for compatibility factors. And the fact that crosses between ICS 1 and ICS 6 and ICS 44 all give self-compatible progeny whilst the cross, ICS 1 × ICS 45, gives all self- and cross-incompatible progeny, suggests that self-compatible trees may be of two kinds, homozygous at two different loci. It becomes obvious that the simple hypothesis of Knight and Rogers, involving as it does only the S locus, is inadequate to explain the results of hybridising ICS 1 with ICS 45. The results obtained from crosses between ICS 1, ICS 45 and ICS 60 point quite clearly to the fact that two other loci are involved besides the S locus. These other loci have been called A and B, and their role has been regarded as being concerned with the production of an "incompatibility precursor" (formed before meiosis), upon which the S-alleles act to impart specific antigen-antibody properties to the gametes. The A and B loci are assumed to show simple dominance and recessivity; the recessive alleles are inactive. If either locus is in a homozygous recessive condition, the precursor is not produced and the S-alleles cannot manifest their action, having no substrate on which to act; the tree is then self-compatible. Similarly, if the S locus is inactive because of amorphism and becomes homozygous for the amorph, the tree will be self-compatible. The clones, ICS 1, ICS 6 and ICS 44, are believed to have the constitution, $AABBS_{f\cdot f}$, where S_f is an amorph of the S series. ICS 45, on the other hand, has been given the constitution, $AabbS_{a\cdot a}$. All of the results obtained from investigations on the 14 groups of progeny can be explained by hypothecating the following genotypes for the parent clones :—

ICS 1, ICS 6, ICS 44	$AABBS_{f\cdot f}$	
ICS 45	$AabbS_{a\cdot a}$	
ICS 60	$AaBBS_{a\cdot f}$	where $S_a=S_b=S_c>S_d>S_f$
ICS 16	$AABBS_{a\cdot f}$	
SCA 12	$AABBS_{b\cdot d}$	
SCA 6	$AABBS_{b\cdot c}$	

The course of events thought likely to occur in cacao is as follows :—

(1) Completely non-specific precursor bodies are formed in diploid cells by the action of the A and B loci, and come to lie in the cytoplasm.

(2) In pollen mother cells the non-specific precursor bodies are given some small modification by the dominant S-allele to promote them into antigen precursors. The recessive allele also present is unable to produce its own antigen-precursor because the dominant allele competes successfully with it for the non-specific precursor. In megaspore mother cells the dominant S-allele acts upon the non-specific precursor to induce the promotion of an antibody precursor, suppressing the recessive allele by competition.

(3) After meiosis, the gametic nuclei carrying the dominant allele further endow the antigen- and antibody-precursors with full antigen and antibody properties which are specific for that allele. Haploid nuclei carrying the recessive allele cannot further modify the antigen- and antibody-precursors because these have been "stamped" with the character of the dominant allele before meiosis. The specific antigen and antibody substances lie in the cytoplasm surrounding the male and female gamates; if both are present non-fusion results.

(4) Where alleles of equal dominance are together both precursors are formed before meiosis. Both S-alleles give a preliminary " stamping " to the non-specific precursor, so that after meiosis the two types of haploid nuclei have each their own substrate on which to carry out their own specific development of full antigenic and antibody characters.

This somewhat complicated chain of events appears necessary, because if haploid gametes reacted with a completely non-specific precursor according to their S-allele content after meiosis, there is no good reason why both alleles should not produce their specific antigens and antibodies, since the dominant and recessive alleles would now be spatially separated. The suppression of the recessive allele by its dominant partner must occur before meiosis, yet the cytoplasm of all gametes cannot be identical in properties.

The reason why three types of self-incompatible tree have been found is now clear. The two S-alleles present must either be (1) both different (2) both different, but of equal dominance (3) both alike. It can be shown that the first case will usually be the most commonly occurring one.

Analyses of genotype and allele frequencies in cacao populations containing only active S-alleles reveal that recessivity always enjoys selective advantage. In any population of self-incompatibles in equilibrium, the bottom-recessive homozygote is the most frequently encountered genotype, and the bottom-recessive allele predominates. If a new bottom-recessive allele appears in a population, the population assumes new stable genotype proportions wherein the homozygous bottom-recessive is again the predominant genotype and the bottom-recessive allele the most abundantly represented. Analyses further show that if the amorphic S_f allele penetrates into a population which contains only self-incompatible trees, the selective advantage becomes so overwhelming that the population, unable to attain a new state of equilibrium, comes eventually to contain only self-compatible elements, the S-alleles being eliminated one by one in order of dominance. This process is taking place in the Trinidad cacao population, the principal constituents of which now are $S_{a \cdot a}$, $S_{f \cdot f}$ and $S_{a \cdot f}$ (in that order of increasing relative abundance), where S_a was the bottom-recessive before the penetration of the amorphic S_f allele ; it can be only a matter of time for the eventual elimination of the S_a allele to be achieved as has already been, very largely, the fate of any original alleles dominant to S_a. The largest population element being $S_{a \cdot f}$ (a 25% non-fusion self-incompatible genotype), it is not surprising that no case of cross-compatibility between Trinidad self-incompatibles has yet been discovered. ICS 90 (the only 100% non-fusion self-incompatible amongst the 100 ICS clones) must be a representative of the homozygous $S_{a \cdot a}$ group and would be expected not to cross with any of the $S_{a \cdot f}$ group—which is, indeed, the case.

Discussion

In cacao the incompatibility reaction is delayed until male and female gametes have met in the embryo-sac. Once fertilised, the ovules appear not to be receptive towards any later penetrating pollen-tubes (even if these bring compatible gametes), so that a flower which has been incompatibly pollinated represents a wastage of female generative material. Other species which discriminate against incompatible pollen-tubes in the style have the advantage of conserving their female gametes (for as long as the flower is receptive) until such time as compatible pollen is placed on the stigma. Because the cacao mechanism introduces wastage of female gametes, some authors have claimed that the term " incompatibility " can scarcely be applied in this " primitive " case, their contention being that conservation of female gametes until compatible fertilisation is achieved is a fundamental requirement of an incompatibility mechanism. Whatever the merits of this view may be, the primitiveness or otherwise of the cacao mechanism cannot be discussed without reference to the biology of the species. As anyone familiar with the tree will know, cacao flowers profusely yet produces only a small number of large fruits. From studies made by the author, it would appear that even on self-compatible trees (able to set fruit with pollen from any other cacao tree as well as with their own) only one ovule in every 500 ever has the chance of developing into a seed. The other 499 are lost through an extremely low level of pollination and through physiological-wilt loss of immature fruits. Evidence exists, indeed, to show that if pollination were more efficient than it actually is, then wilt losses would be more severe, so tending to keep the ratio of viable zygotes to ovules reasonably constant. In the circumstance of extremely heavy loss of female gametes from causes other than incompatibility, the assertion of Knight and Rogers (1955) that " to achieve incompatibility in cacao there is an enormous loss of flowers " cannot be justified. With its profuse flowering habit, there is no need for " sieving out " incompatible pollen tubes in the style, for no advantage would be gained by leaving a flower open to a second, and perhaps compatible, pollination when so many other still-unpollinated flowers are available, on any of which the successful pollination could be made as an alternative. No grounds exist for regarding the incompatibility mechanism as primitive merely on the score of loss of female gametes, the great majority of which, in any case, are destined never to be mated. The restriction of the site of the incompatibility reaction to the style might be essential for the survival of species which are not perennial and which do not flower profusely, but such is not the case for cacao.

Incompatibility mechanisms promote outbreeding, with its attendant advantages of maintaining genetic variability on which the environment acts to select those genotypes most fitted. The incidence of incompatibility would have considerable selective advantage in the early evolutionary history of a species but, later, when adaptation to the environment had been achieved, the continuing necessity for outbreeding would lessen. Self-compatibility, allowing inbreeding, would not be a disadvantage in a species when once adapted to its environment ; indeed, if a species is eventually to achieve a wide-spread distribution a reversion to the self-compatible condition, sooner or later in its evolutionary history, would appear almost to be essential. The extension of a self-incompatible species in space cannot be achieved unless groups of plants, in which cross-compatibility exists, are simultaneously established in successive order from the centre of origin. Even isolated self-compatible plants, however, could contribute to the outward spread of the species. It is perhaps not surprising, therefore, to find (as far as our scanty knowledge allows) that cacao trees growing near the hypothetical centre of origin are all self-incompatible, whilst those at the fringes of its distribution are predominantly self-compatible. Cheesman (1944) places the centre of origin of *T. cacao*

on the eastern equatorial slopes of the Andes and cacao collections made in the vicinity have yielded only self-incompatible types ; further removed, in the lower reaches of the Amazon and in Colombia and Central America, self-compatible trees are encountered.

Two methods of achieving the self-compatible condition in cacao are now known. Mutation of the S locus to an inactive amorph, or even to a condition where the mutant allele had a greatly reduced activity, would lead to compatibility once homozygosity had been attained. Similarly, mutation at either or both of the A and B loci to an inactive recessive mutant would create the compatible condition, when homozygous, by blocking the formation of the non-specific incompatibility precursor. Mutations of either type, once produced, would always be conserved in a cacao population provided that the flower in which they arose was compatibly cross-pollinated. Amongst the resulting progeny at least one genotype would be heterozygous at the mutated locus, and would still be self-incompatible. When this tree was compatibly cross-pollinated and gave progeny, half of the offspring would be heterozygous and half homozygous for the wild-type allele ; but because sib-compatibility is usual in cacao, crosses between the heterozygous progeny would give a proportion of new progenies now homozygous for the mutant allele. Such trees would be self-compatible ; their proportion in an isolated population would ever increase until all trees were self-compatible.

From extremely limited available evidence, it might appear that mutation at the S locus (to the inactive amorph, S_f) is characteristic of the Trinidad self-compatible tree. ICS 45, a tree which is homozygous for an inactive mutant at either of the A or B loci, has strong Central-American criollo affinities. Possibly, then, the two self-compatible types have arisen in rather widely-separated geographical areas.

Why incompatibly pollinated cacao flowers absciss is not known. In the great majority of incompatible matings there are present some 75% of the fertilised ovules in which the embryo-sac content cannot be distinguished from that of ovules in compatible matings ; gametic fusions have taken place and endosperm divisions have been initiated. Mature fruits on cacao trees may often be found to contain seeds which number less than half the available ovules, so that the abscission of incompatibly pollinated flowers appears in some way to be connected with the presence of non-fusion ovules in the ovary. Note has already been made that nucellar cells lining the embryo-sac in such ovules often show a condition of cellular degeneration ; possibly, then, the breakdown products which might diffuse from non-fusion ovules interfere with the development, in the normal ovules, of a concentration of growth-promoting substances large enough to prevent the abscission mechanism from coming into operation. The abscission of incompatibly pollinated flowers is essential to the success of the incompatibility mechanism, for if the flowers developed which had a proportion of normal ovules in the ovary the formation of the homozygous top-dominant would alone be discriminated against, and the advantages of outbreeding would be lost.

The incidence of incompatibility in cacao has considerable impact upon a breeding programme in that crop, not all of it adverse in effect. Where self-incompatible trees are absent in a population, the continuing inbreeding made possible tends to reduce genetic variability, so that a programme of selection of superior genotypes for clonal propagation will have less chance of success than where the selectionist can work in a population whose variability is maintained by the incidence of self-incompatibility. Practical experience of selection in Trinidad and in Grenada shows that many of the most promising selections are self-incompatible ; if, as is here the case, self-compatible elements are also present in the populations, the self-incompatible selections are likely all to have the same dominant allele and will, therefore, also be cross-incompatible. Monoclone plantings of self-incompatible selections, or plantings of mixtures of self-incompatible clones, would, therefore, have to be avoided.

Attention has recently been focused on the hybridisation of materials of widely different geographical provenance. Seedling progenies arising from crosses between Amazon and Trinidad clones, for example, show considerable promise for vigour and bearing. Cross-compatibility between such parent plants is usually the rule, for different sets of S-alleles are involved. Although all progeny are individually self-incompatible, there is usually cross-compatibility between groups of them, so that the planting of compatible pollinators amongst the seedling trees is not essential. The only case which will lead to complete absence of cross-compatibility between sibs is one which involves a parent homozygous for an S-allele, dominant to those of the other parent (e.g. $S_{2\cdot2} \times S_{3\cdot4}$ gives $S_{2\cdot3}$ and $S_{2\cdot4}$ progeny, and these are cross-incompatible because of the common dominant, S_2). Where a cross is made involving a self-compatible Trinidad clone, it would always be possible to breed self-compatible progeny in F_2, unless the Amazon parent were homozygous for an S-allele ; and, since nearly all Trinidad self-incompatibles carry the amorphic S_f, so again, in F_2 self-compatible genotypes would emerge, provided the Amazon parent was heterozygous. The plant breeder's ability to effect back-crosses with the Amazon and Trinidad parents will depend upon the dominance relationships of the parental alleles. If the Trinidad parent is self-compatible, back-crosses to this parent are always possible ; the same is true if the Trinidad parent has an S-allele (with S_f) which is recessive to both Amazon alleles. Where the Trinidad allele is dominant to the Amazon alleles, only half of the progeny will back-cross to the Trinidad self-incompatible parent ; if it lies intermediately in dominance between the Amazon alleles, the probability that any progeny will back-cross to the Trinidad parent is now $\frac{3}{4}$. For back-crosses to the Amazon parent, the chance of success is never greater than $\frac{3}{4}$ and is more likely to be only one-half.

Self-incompatibility has its advantages in the establishment of seed gardens for the production of hybrid seed between self-incompatible but cross-compatible parents. If, in an area isolated from other cacao, a mixture of Amazon and Trinidad clones is planted, then all pods borne on the Amazon material will be cross-pollinated with the Trinidad clone, since the former is self-incompatible. If the Trinidad parent is also self-incompatible, then all pods produced in the seed garden will arise from cross-pollination only.

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Nutritional Factors associated with the Development of Mealybugs on Cacao

by

R. G. Fennah

Introduction

In this study an attempt is made to account for the distribution of a mixed population of mealybugs on young cacao plants subjected to different treatments during growth in in the field or in the greenhouse. The stimulus for its inception was provided by observations of failure on the part of the insects to exploit with equal success substrates which superficially appeared to be equally suitable. Although some of the relationships reported below refer only to the conditions in which the data were obtained, or to conditions closely similar, the hypothesis put forward to accommodate the observations implies that the mealybug species studied are dependent for their development on appropriate conditioning of their host substrate, and that, in cacao, such conditioning is partly determined by factors which are independent of the presence of the insect.

The mealybug population

An account of the mealybugs infesting cacao in Trinidad has been given by Kirkpatrick (1952), and the list of species includes *Planococcus citri* (Risso), *Dysmicoccus brevipes* (Ckll.), an apparently undescribed species close to *D. brevipes*, *Pseudococcus adonidum* (L.) (=*P. longispinus* Targ.), *P. comstocki* Kuwana, *P. sp.* near *comstocki* Kuw., *Ferrisiana virgata* (Ckll.) and *Puto barberi* Ckll.

In the field observations reported below the mealybug population, on the basis of the samples taken, appears to have been overwhelmingly dominated by *P. citri*, and it is accordingly to this species that the conclusions reached principally apply. In the greenhouse *P. citri* was invariably the first species to become abundant, but after a time became reduced by parasitoid attack, whereupon *Ferrisiana* became dominant: other species were absent or rare. From the standpoint of feeding behaviour there was little difference between the species present, but the tendency of *Ferrisiana*, noted by Kirkpatrick, to occur on cacao leaves more often than most other mealybugs was evident on greenhouse material.

In the field plot where observations were made, almost all the mealybug colonies were attended by ants, and of these *Wasmannia auropunctata* (Roger) was the most common. In the greenhouse ants were sometimes present. *Ferrisiana* was not seen to be attended.

Distribution of mealybugs on host plant

In the laboratory *P. citri* may multiply until the colony is large enough to cover much of the surface of a " quailing " potato: in the field, in appropriate circumstances, they may form an almost continuous felt over much of a large cacao pod as it hangs exposed on the tree. But they have not been observed to form a blanket cover over the subaerial parts of an entire cacao plant, however small it might be, and heavy infestation of terminal shoots has been observed only under conditions of enrivonmental duress in the greenhouse. Indeed, it is remarkable that an insect with so catholic a taste in the matter of host plants as *P. citri* should be so eclectic in its choice of feeding sites on any one of them. On cacao this species, and the others listed above, may be found on terminal buds, buds in leaf axils, flower cushions, the walls of cherelles, and sometimes on wounds in the bark; on conducting tissue such as green stems, petioles, peduncles and major leaf-veins; and in special circumstances, on the lower side of a leaf in proximity to a point of injury, and on heavily shaded juvenile shoots, or on maturing pods, borne on " retarded " trees.

Distribution of mealybugs in an experimental plot of cacao trees

Mealybug incidence was followed for two successive years on trees $2\frac{1}{2}$ to $3\frac{1}{2}$ years old in the Shade and Fertiliser experiment at the I.C.T.A. A description of this has been given by Evans and Murray (1953) and in it three ICS clones were grown, with replication, under eight different fertiliser treatments and five degrees of light intensity. The layout and treatments are shown in Figs. 1 and 2. It is to be noted that at this age the trees were still small, the canopy had not closed in, and flowering and fruit-bearing had only recently begun.

The mealybug population was estimated by means of a five-minute scrutiny of each tree. The data were recorded under " infestations " where one mealybug or more at a given spot constituted one infestation and also under " colonies " where an agglomeration of individual insects surpassed half an inch in length or width. Each large agglomeration was accordingly entered both as an " infestation " and as a "colony," but in the final gradation of incidence was counted as a " colony." The entries were next grouped into " light," " medium " and "heavy " degrees of incidence, where " light " meant one to three " infestations " on a tree, " medium " meant four to seven " infestations," or alternatively a single " colony," and " heavy " meant more than seven " infestations " or one " colony," and each tree was assigned to a class determined by its infestation rating. The classes corresponded to the three degrees of infestation with an additional category added to accommodate uninfested trees. On the " heavily infested " trees the presence of mealybugs was immediately discernible, but their numbers were not of such a magnitude as to call for the use of insecticides in order to protect the

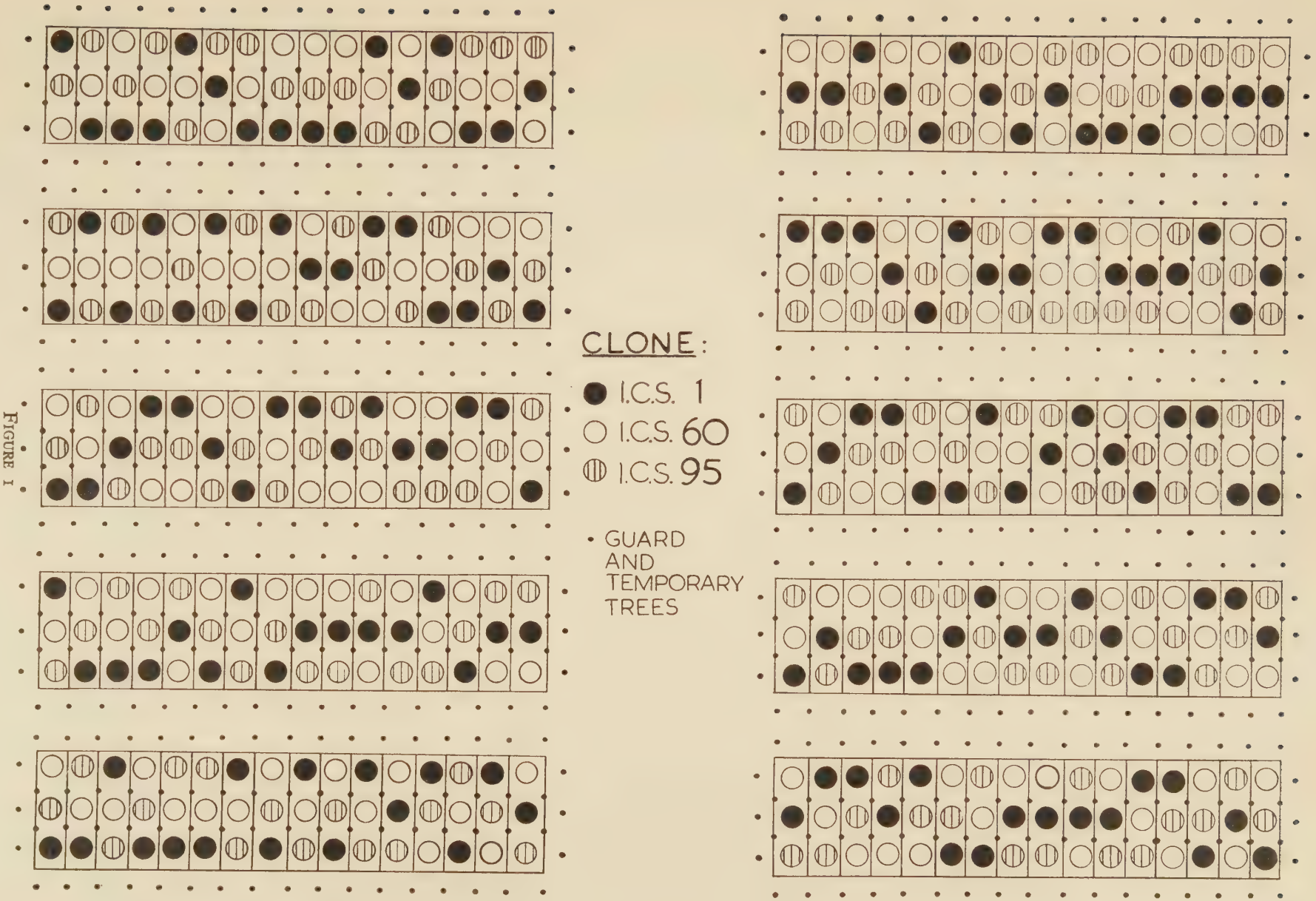

FIGURE 1

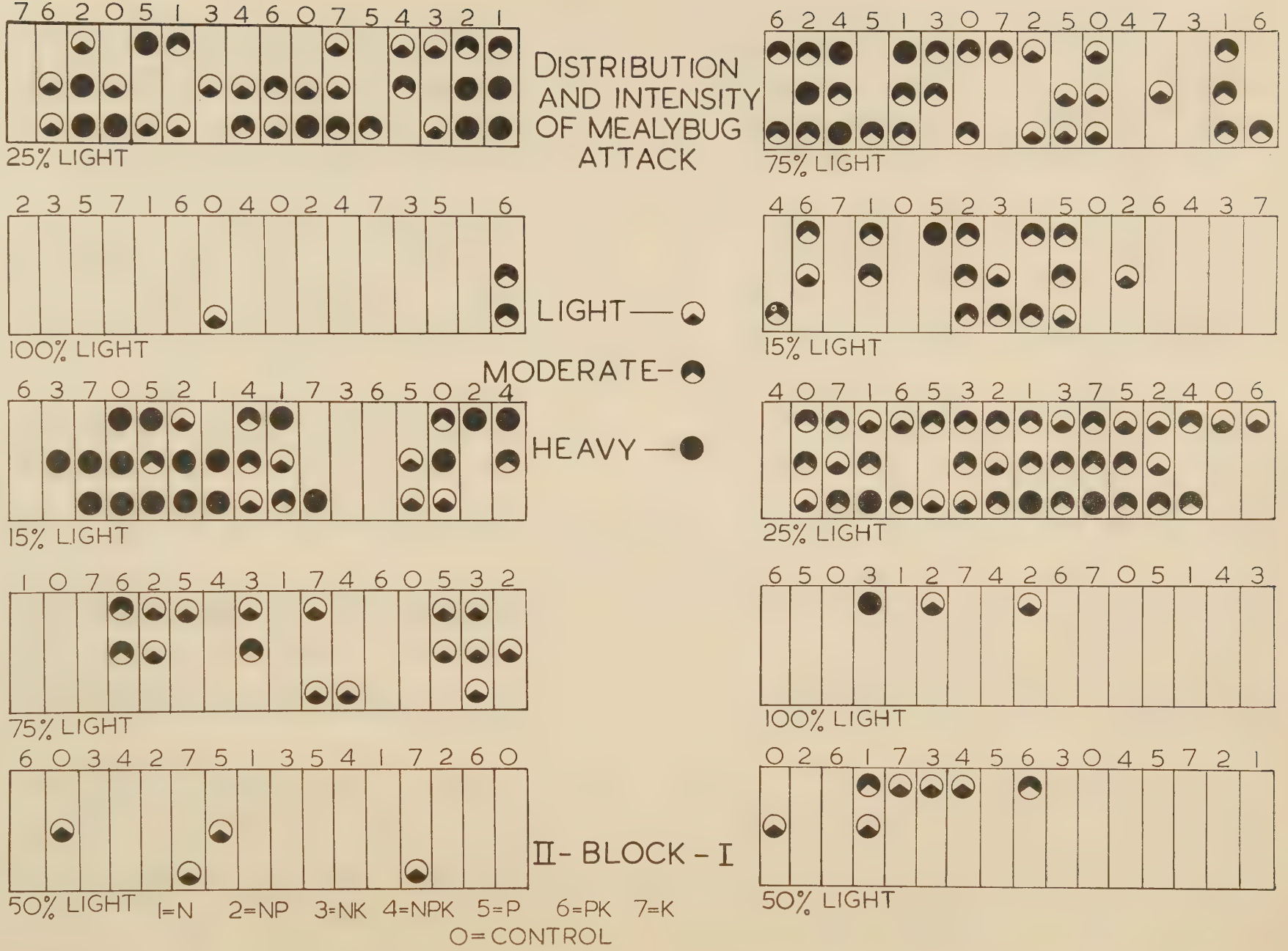
7 6 2 0 5 1 3 4 6 0 7 5 4 3 2 1
25% LIGHT
DISTRIBUTION AND INTENSITY OF MEALYBUG ATTACK
6 2 4 5 1 3 0 7 2 5 0 4 7 3 1 6
75% LIGHT
2 3 5 7 1 6 0 4 0 2 4 7 3 5 1 6
100% LIGHT
LIGHT —
MODERATE —
HEAVY —
4 6 7 1 0 5 2 3 1 5 0 2 6 4 3 7
15% LIGHT
6 3 7 0 5 2 1 4 1 7 3 6 5 0 2 4
15% LIGHT
4 0 7 1 6 5 3 2 1 3 7 5 2 4 0 6
25% LIGHT
1 0 7 6 2 5 4 3 1 7 4 6 0 5 3 2
75% LIGHT
6 5 0 3 1 2 7 4 2 6 7 0 5 1 4 3
100% LIGHT
6 0 3 4 2 7 5 1 3 5 4 1 7 2 6 0
50% LIGHT
II - BLOCK - I
1=N 2=NP 3=NK 4=NPK 5=P 6=PK 7=K
0=CONTROL
0 2 6 1 7 3 4 5 6 3 0 4 5 7 2 1
50% LIGHT

FIGURE 2

trees. The distribution of mealybug incidence, as estimated above, is shown in Fig. 2.

The design of the Shade and Fertiliser experiment permitted the relationship between mealybug incidence and the effect of the experimental treatments to be assessed directly. One additional factor, however, could not be so measured. This was the effect of differences in degree of root aeration of the host plants on the development of infestation. All trees in the experiment received the same amount of rain, but the soil in which they grew varied in texture from gravel to silty-loam. This resulted in tree-sites being relatively " good " or " bad " for growth of the trees at different times of the year, with a distinct tendency for the worst soil environment during the rainy season to be associated with the more finely-textured soil. The distribution of trees, defined as " superior " or " inferior " in relation to the mean level in a number of characteristics of growth, was determined (Fig. 3) and the site of each tree was associated with certain gross physical characters of the soil by reference to a map prepared by Smith (1954) (Fig. 4).

Notwithstanding the comparatively mild nature of the total infestation recorded, the results are clear cut. The incidence of mealybugs was significantly heavier in the shade plots than in the light, and was negligible on trees grown in full daylight. Trees which received nitrogenous fertiliser were significantly more heavily infested than those which did not, whereas trees which received potash were less infested than those which did not. There was no difference in infestation between trees which received, or did not receive, phosphate. Differences between the clones did not attain the level of significance.

The infestation level of mealybugs on trees of the clone ICS 1 was negatively correlated with the vigour of the trees : this, in turn, was negatively correlated with the depth of gravel below the soil surface. Smith (1954) established a similar relationship on this experiment between depth of gravel below the soil surface and yield per tree. The corresponding data with regard to the clones ICS 95 and ICS 60 showed a similar trend, but the differences did not attain the level of significance chosen.

The results of a second survey of mealybug distribution in the experiment did not differ significantly from those of the first, and were, in fact, closely similar.

Observations were made for a period of six months of insect attack on rooted cacao cuttings grown in " sterile " media and under a nutrient regime deficient in one of all the known essential nutrient elements. The media in which the plants were grown comprised (*a*) distilled and appropriately demineralised water through which air was bubbled, (*b*) acid-washed quartz sand with particle size of 3 mm. and, for seven treatments, (*c*) vermiculite. The greenhouses in which the plants were grown became drier and hotter during the day than is ever experienced outside. In the house where the plants in sand and vermiculite were grown the midday temperature varied between 98°F. and 104°F., while the relative humidity at the same time varied between 58% and 48%. This resulted in foliar tissues being subjected to a greater water stress than is normally experienced, even in the series of trees which received a complete treatment.

Each of the treatments given was expected to produce some degree of abnormality in the metabolism of the plant, and observations were maintained to determine whether the changes induced were accompanied by differences in infestation by mealybugs. In the following table (Table I) a dash indicates that no mealybugs were found at any inspection on any of the plants receiving the treatment listed, the symbol " I " indicates that one or two mealybugs were found, but occurred singly, while a plus sign indicates that an unmistakable build-up of population had occurred. For present purposes only entries in the last category are regarded as important.

In the conditions provided, there was an evident multiplication of mealybugs (*P. citri* and *F. virgata*) on plants deprived of copper, cobalt, zinc and boron. The case of sulphur is of interest, since these plants, as noted by Evans (1953, p. 56) were conspicuously infested with a miscellany of pests (which included *Asterolecanium*, aphids, *Coccus* sp., *Selenothrips* and Red Spider). Mealybugs were, however, absent from the water culture, and only present as single specimens on plants in sand culture. Yet on the sulphur deficient material in vermiculite they abounded. In view of the foliar symptoms present, and of the comment of Evans (1953 : 60) that zinc deficiency was readily induced by over-watering the grade of vermiculite used in this experiment, it is believed that the development of mealybugs on these plants was associated in part with zinc deficiency.

Observations were also made for a period of four months of insect attack on rooted cuttings, grown in a " complete " potting soil in the greenhouse, to which dressings of certain minerals were given, one at a time, in series of increasing concentration. Cuttings which received a copper sulphate dressing, for example, were given either a basic dressing, or twice, or three times or four times this quantity : those which received aluminium sulphate were given either the basic dressing, or three times or five-and-a-half times this amount. The plants were watered normally. Midday humidity in the greenhouse was much lower than outside, being about 50% RH. Under the régime given, the dressings exerted their maximum effect during the first three months, after which growth conditions appear to have reverted almost to normal as far as mineral uptake was concerned.

TABLE I

Nutrient element omitted	Nil	Mag-nesium	Copper	Nitro-gen	Phos-phorus	Potas-sium	Cal-cium	Sul-phur	Boron	Zinc	Man-ganese	Cobalt	Molyb-denum
Water culture ...	—	—	+	—	—	—	—	—	+	+	—	+	I
Quartz sand	I	—	+	—		I	I	I	I	+	+	+	I
Vermiculite	I	+		—	—	—		+	—				

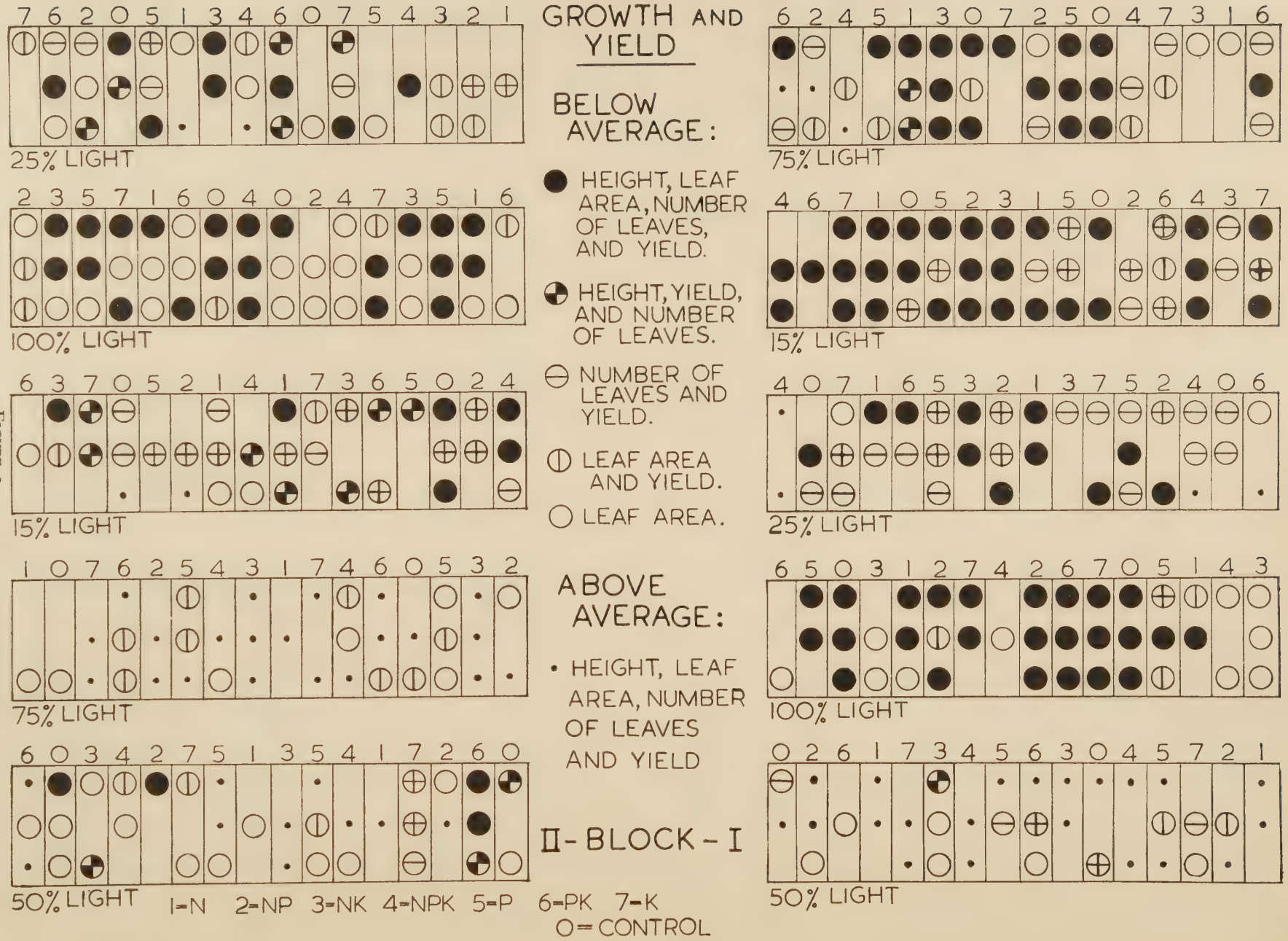
RELATIVE GROWTH AND YIELD
BELOW AVERAGE:
HEIGHT, LEAF AREA, NUMBER OF LEAVES, AND YIELD.
HEIGHT, YIELD, AND NUMBER OF LEAVES.
NUMBER OF LEAVES AND YIELD.
LEAF AREA AND YIELD.
LEAF AREA.
ABOVE AVERAGE:
HEIGHT, LEAF AREA, NUMBER OF LEAVES AND YIELD
II-BLOCK-I
7 6 2 0 5 1 3 4 6 0 7 5 4 3 2 1
25% LIGHT
2 3 5 7 1 6 0 4 0 2 4 7 3 5 1 6
100% LIGHT
6 3 7 0 5 2 1 4 1 7 3 6 5 0 2 4
15% LIGHT
1 0 7 6 2 5 4 3 1 7 4 6 0 5 3 2
75% LIGHT
6 0 3 4 2 7 5 1 3 5 4 1 7 2 6 0
50% LIGHT
1=N 2=NP 3=NK 4=NPK 5=P 6=PK 7=K
0=CONTROL
6 2 4 5 1 3 0 7 2 5 0 4 7 3 1 6
75% LIGHT
4 6 7 1 0 5 2 3 1 5 0 2 6 4 3 7
15% LIGHT
4 0 7 1 6 5 3 2 1 3 7 5 2 4 0 6
25% LIGHT
6 5 0 3 1 2 7 4 2 6 7 0 5 1 4 3
100% LIGHT
0 2 6 1 7 3 4 5 6 3 0 4 5 7 2 1
50% LIGHT

FIGURE 3

Depth from soil surface to upper surface of gravel
Inches
0 - 6
6 - 12
12 - 18
18 - 24
24 - 36
36 - 48
> 48
II - BLOCK - I

FIGURE 4

The history of the mealybug component of the insect population present is given in Table II, where the symbols used have the same meaning as in Table I. The rate of application is given as multiples of the basic dressing, which forms the first row of the table.

The most evident effects of the treatments on the appearance of leaves have been described and illustrated by Evans (1953, p. 66 and Pls. 12, 13) and it is obvious that in the conditions which prevailed very appreciable metabolic derangement ensued. Yet these plants, with the exception of those receiving the lowest dose of aluminium sulphate, and those receiving boron, remained remarkably free from mealybug attack. The attack on the young shoots of the plants receiving the basic dressing of aluminium sulphate increased for a time, then declined before the population had become large. The plants receiving the higher dressings of boron, however, were extremely retarded or were killed, and at the "double dressing" level became heavily infested with mealybugs. Above this treatment level growth remained almost at a standstill until the plants died, and as leaves were lost they were not replaced by new growth.

Two sets of rooted cuttings of ICS 1, each comprising ten plants, were grown in a neutral potting soil in a greenhouse. Five trees were watered normally with "hard" (calciferous) water, five were watered as sparingly as possible with the same type of water, five were watered normally with water weakly acidified with sulphuric acid, and five were watered sparingly with the acidified water. The daily maximum temperature and minimum humidity were markedly different from those recorded in the Shade and Fertiliser experiment. The average ranges between December and January are shown below (Table III). The development of mealybug populations in these conditions was followed.

The results were clear cut. After four weeks a substantial population of *Pseudococcus citri* had built up on all the plants normally watered with "hard" water, but had not developed on the remainder. After sixteen weeks *P. citri* and *Ferrisiana virgata* were common on all the normally watered plants, and were sparsely present on some of the sparingly watered plants and not on others. The sparingly watered plants made slower growth than the normally watered plants, and the average leaf size, at this age, was smaller. Chlorosis of young leaves, indicative of a deficiency of "labile" iron, developed in the normally watered plants, but only in the "acidified water" series after the soil had become compacted.

Finally, two sets of rooted cuttings of ICS 1, each comprising sixteen plants, were grown in neutral potting soil

TABLE II

Application rate	Copper sulphate			Manganese sulphate		Ferrous sulphate			Aluminium sulphate			Zinc sulphate			Boron		Sodium chloride		
	Dec.	Feb.	Mar.	Feb.	Mar.	Dec.	Feb.	Mar.	Dec.	Feb.	Mar.	Dec.	Feb.	Mar.	Feb.	Mar.	Dec.	Feb.	Mar.
1.0	—	—	1	—	1	—	1	—	+	+	—	—	—	—	1	—	—	1	—
1.5				—	—												—	—	—
2.0	—	—	—	—	1										+	+	—	—	—
2.5												—	—	1			—	1	—
3.0	—	—	—			—	—	—	—	—	—				—	*			
3.5				—	—														
4.0	—	—	—												*	*			
4.5																			
5.0												—	—	—					
5.5									1	—	—								

* Plant dead, with no sign of mealybug infestation.

TABLE III

Diurnal range of air temperature and humidity in December-January
(rainless days only)

Time (hrs.)	Greenhouse		Shade and fertiliser experiment			
			100% light plot		15% light plot	
	Temperature °F.	RH%	Temperature °F.	RH%	Temperature °F.	RH%
01.00	73	97	68	100	68	100
06.00	72	100	74	97	72	97
09.00	88	65	85	77	80	80
12.00	99	54	80	64	86	73
15.00	94	56	85	80	85	71
18.00	81	85	74	96	73	95
21.00	76	94	72	98	72	98
24.00	74	95	70	100	70	100

in cacao baskets, and were kept with the basket completely and continually immersed in rainwater which was topped up to compensate for evaporation. One set was placed in the greenhouse, the other outside among cacao where lateral shade was dense and overhead light was approximately 75% of full sunlight. The plants were " inoculated " with crawlers of *P. citri*. After four weeks the plants in the greenhouse were showing signs of general starvation, and were sparsely infested with mealybugs or not at all infested. At the same time the plants outside, which had made rather slow but steady growth, were green and were virtually free from mealybugs. After twenty weeks the plants in the greenhouse were very seriously debilitated : the foliage had become reduced to a few sparse undersized leaves on each plant. Notwithstanding repeated inoculations where it appeared necessary, mealybugs remained very few, were absent from most of the plants and did not produce a heavy infestation on any. The plants outside in the same period had developed symptoms of general starvation, and, indeed, continued to decline steadily over a period of more than a year. An occasional isolated mealybug was noted but never founded a colony, and in general the plants remained uninfested.

Crawlers for infesting the above plants were bred in a separate compartment of the greenhouse. Rooted cuttings were established in potting soil, supplied with nitrogen in the form of sulphate of ammonia, and watered liberally with " hard " water. In these circumstances mealybugs bred rapidly, but after a time the initial colonies of *P. citri* became depleted by parasitoids and supplanted by *F. virgata*.

Discussion

In suitable environmental conditions, and in the absence of interference, an organism, when supplied for an adequate time with an abundance of its normal food, multiplies, and its progeny in due course tend to distribute themselves more or less evenly over the available food-yielding area. In comparable circumstances the behaviour of *Planococcus citri* is similar, as when, for example, it is reared in the laboratory at room temperature on tubers of potato. This species, like *Ferrisiana virgata* and, indeed, all the named species of mealybugs so far found on cacao in Trinidad, has a wide range of host plants which differ substantially in their external morphology, environmental tolerances and time of flowering and fruiting. In view of this it might be surmised that the dietetic needs of so general a feeder can readily be met, and that failure to colonise an apparently suitable substrate after a sufficient period of time is to be ascribed to the repressive effect of enemies or pathogens or to the unsuitability of the available eco-climates. Nevertheless, if an attempt is made to account for the distribution of mealybugs as observed in the shade and fertiliser experiment by invoking only these factors, serious difficulties are encountered.

The principal enemies of *P. citri* in Trinidad appear to be coccinellid and cecidomyiid predators and the encyrtid parasitoid *Leptomastix dactylopii* Howard (Kirkpatrick 1953, p. 124). In the greenhouse the last more than once effectively checked the development of *P. citri*, but never to such an extent that uninfested plants were not liable to infestation by " crawlers." In the shade experiment the parasitoid and predators may well have strongly depressed the potential population of mealybugs, but as mealybugs occurred in every plot of the experiment, it is to be expected that the repressive effect was exercised evenly within each plot, and that the mealybug population was not selectively " spared " by its enemies at the pickets receiving specific fertiliser treatments, or situated on deep silty-clay. In the greenhouse the situation was sufficiently clear : predators were totally absent, and *Leptomastix* was the only obvious enemy present. This attacks adults and third instar nymphs. There was no biological pressure on the " crawlers," and their failure to establish themselves on certain plants with the same success as on others, or not at all, cannot be credited to the activity of natural enemies. If it is accepted that the distribution pattern of mealybugs noted could not have been brought about by a parasitoid which was free to attack its host on any tree, then it must also be assumed that it is unlikely to have been brought about by the incidence of a pathogen, even if it is assumed that a pathogen was present.

If an explanation of the observed distribution is attempted along ecoclimatic lines, by postulating that in certain micro-environments on the host mealybug reproduction and the successful establishment of crawlers can take place, whereas at others conditions are physically unsuitable, then difficulties arise in defining such environments. In Table III the ranges of daily temperatures and humidities are given : they contrast appreciably, and in every case they represent conditions close to the points where mealybugs were found. It is clear that there is a larger difference between the subaerial environment of a mealybug-infested tree in 15% light and that of a mealybug-infested rooted cutting in the greenhouse than there is between two adjacent trees, one infested, the other not, in the same plot. If differences from spot to spot in the subaerial environment are to be admitted as an effective factor in the present problem, they may most legitimately be invoked to account for gross differences between plots. Yet even so it is not evident how they could have directly affected the mealybugs though it is possible to show how they have affected the host plant.

It has been established that in the shade experiment, for a period of two years, mealybug populations were higher on trees which were below average in yield and general growth, and that such trees tended to occur in patches of soil with certain physical characteristics. If, in so small an area, the distribution of mealybugs is determined in part by that of a specific texture of soil, while the distribution of " unthrifty " cacao trees is similarly determined, it is legitimate to assume that the soil texture influences the development of the plants, and that these in turn affect the development of the mealybugs.

Although the effects of variation in soil texture could be detected in the tree by appropriate measurements, there was no superficial qualitative characteristic by which a leaf or pod could be identified on inspection as having come from an unthrifty tree. Foliar symptoms of malnutrition did not appear in the heavily shaded plots, and, as far as leaf colour is concerned, there was more variation between the flaccid " flush " leaves and the mature leaves of a single tree than between mature leaves of adjacent trees of different clones. There were, in short, no differences in tone or colour between trees of a single plot which could be considered as possible " guides " to wandering mealybugs, or as possible stimuli to settle down.

The effects of the fertiliser treatments applied in the shade experiment on the growth rate and yield of trees, and on leaf composition have been reported by Evans and Murray (1953) and Murray (1953, 1954, 1955).

It has been shown that the application of nitrogen, potassium or phosphorus, singly or in combination, led to the accumulation of a larger amount of the corresponding elements in the leaf, as compared with the untreated control. It has also been shown, however, that both the rate of growth and the amount of fruit-production, and by extension, the demand for these nutrients on the part of the plant, are profoundly influenced by the light intensity under which the trees are grown.

Lemée, quoted by Poncin (1958), has shown that at Bingerville the rate of carbon dioxide assimilation increases sharply with increase of light intensity to a level of about 25% of full sunlight, then increases much more slowly at higher light intensities. Murray (1953) found that growth of cacao in Trinidad was optimal at a light intensity of 50% of full sunlight until mutual shading occurred as the canopy thickened. The writer (Fennah 1953) has shown that the rate of metabolism is slower in 15% of full sunlight than in full sunlight, and a similar conclusion has been drawn by Murray (1953) from data of the Shade and Fertiliser Experiment.

Murray has also established that trees grown in full sunlight periodically suffered nitrogen starvation even on plots to which nitrogen had been applied : conversely it was shown that trees grown at the lowest light intensities accumulate reserves of nitrogen and potash which are promptly utilised for growth and fruit-production if the light intensity is increased (Murray 1955).

The environmental factor which on certain patches in the Shade and Fertiliser Experiment adversely affected growth of trees was considered to be subaeration of roots in the wet season as the result of impeded drainage (Evans and Murray 1953, p. 70, Smith 1954). On neutral silty clays of adequate calcium status such waterlogging appears to promote uptake of calcium or to depress uptake of potassium, or to do both : the broad effect, as reflected by foliar analyses, is to induce a nutrient status in the young leaf which is similar to that normally found only in old or senescent leaves. In severe cases metabolism is further deranged by the immobilisation of certain minor elements in the plant in forms unavailable for utilisation. Havord (1955) investigated conditions associated with lime-induced chlorosis of cacao, and, in a personal communication to the writer, reported that all cacao plants grown in overwatered calcareous soils became infested with mealybugs, whereas all those grown in the corresponding lightly-watered series remained uninfested. The growth rate of the overwatered plants was less than that of the lightly watered, and the accumulation of iron in the leaves, apparently in a form unavailable for normal metabolism, was five to seven times as much in the overwatered plants as in the sparingly watered plants. This finding is in accord with experience at I.C.T.A. that mealybugs multiply readily on cacao plants grown in the greenhouse in neutral potting soil under a regime of liberal applications of calciferous water.

The absorption of potassium by plants is antagonistic to that of calcium (Ehrenberg (1919), Shibuya and Torii (1935), Wetzel (1939), Peech and Bradfield (1943)) and this has been verified in cacao (Murray 1957, p. 21). Where the degree of subaeration in relation to the amount of free lime in the soil seasonally approaches the level at which calcium uptake by the plant at the expense of potassium becomes high, it is reasonable to expect that a reserve of mobilisable potassium in the plant will serve in some degree to stabilise metabolism.

It has been established that abundance of mealybugs was associated with pickets which had received nitrogenous fertiliser, as well as with those in plots under the greatest amounts of shade. It has also been shown that the rate at which nitrogen was utilised by the plants in the Shade and Fertiliser Experiment was related to the light intensity under which they were grown.

The essential food requirements of *P. citri*, or of any of the species infesting cacao, have not been investigated experimentally. Insects in general require carbohydrate, organic nitrogen, sulphur and phosphorus, and salts, and if they are unable to synthesise certain substances, such as vitamins of the B group, these also must be present in the diet. Carbohydrate is available to mealybugs in the form of soluble sugars, and nitrogen, sulphur and phosphorus in the form of amino acids. The rate of growth, and perhaps of ovum-production, of a mealybug is governed by the rate at which the insect can assimilate the required nutrients (or their precursors) and the rate at which metabolism can proceed in its own body.

The second factor imposes an absolute upper limit on the rapidity of development, while the first may define the lower.

Gray (1952) has followed the excretion by *D. brevipes* of substances while feeding on leaves or green fruit of pineapple. He found that the number of amino acids detectable in a sample of honeydew increased with the time for which the mealybug continued feeding up to a limit of ten days. He also established that as the period of feeding increased, the amount of excretion of honeydew decreased, and that after six or eight days the quantity of amino acids in the honeydew was fully ten times that at the outset. In one experiment only three amino acids, cystine, aspartic acid and glutamic acid, appeared in the honeydew after the mealybugs had fed for two days : after four days' feeding seven amino acids were obtained, cystine, aspartic acid, glutamic acid, serine, asparagine, glutamine and one unidentified spot. At the end of prolonged feeding it was possible to detect sixteen amino acids in the sample, cystine, aspartic acid, glutamic acid, serine, glycine, asparagine, threonine, alanine, glutamine, tryptophane, valine, phenylalanine, histidine and proline ; three ninhydrin-reacting substances were not identified.

The writer (1955) has referred to the amino acids of fresh cacao leaf tissue, and De Witt (1957) to those of the fresh bean and of the bean after its protein had been hydrolysed during fermentation. Initially, cystine, aspartic acid and glutamic acid are readily detectable, with some asparagine : the hydrolysate of cacao bean protein contains aspartic acid, glutamic acid, glycine, alanine, valine, leucine, isoleucine, serine, threonine, phenylalanine, tyrosine, cystine, methionine, proline, tryptophane, histidine, lysine, and arginine, together with ammonia. Gray considered that most of the amino acids which he found in honeydew represented unutilised amino acids from the cell solutes of the host plant.

It is evident that the history which he gives is in harmony with an interpretation that his mealybugs initially broached fresh or " normal " solutes in relatively turgid cells, and that with the passage of time there was an upsurge in

amino acids derived from the breakdown of protein (either in cells injured by the insect or located elsewhere) and a diminution in hydration of the tissues explored by the stylets. Among the amino acids which have been established as essential for growth of certain insects are arginine, lysine, methionine, leucine or isoleucine. It would seem that all of these cannot be accommodated in the three ninhydrin-reacting substances not identified, so that it cannot be readily assumed that all the nitrogenous requirements of the mealybugs had been met to surfeit level even after ten days of feeding. On the other hand, there is no reason to assume that by this time growth of the mealybugs was being limited by any form of nutrient shortage : it is entirely possible that they can accumulate an adequate amount of an amino acid even when the latter is present in too low a concentration to be detected in a small unconcentrated aliquot by the ninhydrin reaction.

In such circumstances an increase in the total level of nitrogen within the host plant would increase the potential supply of amino acids in the cell solutes, but this would be realised as an actual increase only if nitrogen were not being synthesised into protein as fast as it was assimilated. It has been shown that in full sunlight the demand of the cacao plant for nitrogen is so heavy that a state of nitrogen deficiency may readily supervene ; conversely in heavy shade, " reserve " nitrogen is accumulated. It is, accordingly, possible to account for the associations between high mealybug populations and nitrogenous treatments, and between high populations and heavy shade by assuming that they are the result of mealybugs being favoured in shaded conditions and at pickets fertilised with nitrogen by the absolute nitrogen status and the rate of nitrogen utilisation in their hosts. A similar assumption will account for the absence or rarity of mealybugs on starved waterlogged cacao plants and their abundance on overwatered plants in potting soil supplied with sulphate of ammonia, where both were in a light intensity of about 60% of full daylight.

The discovery of greatest theoretical interest, and of practical importance, is the relationship between mealybug incidence and soil structure noted in the Shade and Fertiliser Experiment. This relationship is determined by the natural " water regime "—the annual rainfall of this area and its distribution—and, at a given level of shade, determines which trees are markedly infested and which are not. If it be assumed that a mealybug, in order to feed, merely has to pierce the tissues of its host plant, and can then feed for days, or indefinitely, on the soluble products of the tissue breakdown resulting from the incision made by the stylets, and perhaps also from the action of its saliva, then it is extremely difficult to account for the existence of this relationship, as the insects on this assumption would be expected to fare equally well in all parts of a plot at any one level of shade.

One of the results of metabolic derangement of the type described in the plots is that tissue breakdown occurs more easily in trees with overwatered or subaerated roots than in others, and such breakdown is associated with an increase in concentration of amino acids in the cell solutes. There is, therefore, no difficulty in accounting for the successful establishment of mealybugs on so many " waterlogged " trees, but it would seem necessary to account for their relative failure on the remainder by assuming that their feeding activities were less efficient in securing an adequate amount of food.

There are many ways in which the metabolism of a cacao tree may be retarded. Those which lead to rapid drying out of the plant, or to depletion of the inflow of mineral nutrients in general, do not appear to be beneficial to mealybugs. Those which do not impair uptake of nitrogen or of water, and do not involve heavy shade, include excess or deficiency of specific nutrient elements. On plants subjected to treatments which bring about such effects it might be expected that mealybug attack would be consistent and heavy. Nevertheless, the results given in Table I do not bear out this supposition. Although only plants in the " minus nitrogen " and " minus phosphorus " treatments remained entirely uninfested, the mealybugs on many of the remainder were extremely few, and could not be regarded as " successful." In contrast to these, however, the colonies on plants in the " minus copper," " minus zinc," " minus cobalt," " minus boron " and perhaps on " minus sulphur " (or " minus sulphur and zinc ") were unquestionably strong. Of the plants themselves the " minus boron " group was the most stunted and deformed.

The results given in Table II are also of considerable interest. The outstanding feature was the remarkable freedom of all trees from mealybug attack, with the exception of those receiving excess boron in almost lethal amounts, and those receiving aluminium sulphate. The plants receiving boron were greatly stunted and debilitated : the remainder grew vigorously and the symptoms of toxicity which were visible in the earlier months of the experiment disappeared. The plants were grown in the greenhouse and were exposed to low daily humidity.

De Witt (1953) has examined the effect of copper, iron, zinc, boron, manganese and molybdenum deficiency on enzyme activity in leaf material, and has found that deficiency in a specific metal lowers the activity of at least one specific enzyme system. Thus polyphenol oxidase is lacking in leaves deficient in copper, catalase activity is lower in cases of iron and zinc deficiency, while low peroxidase activity is associated with deficiency of manganese, boron and molybdenum. His investigation was limited only to the enzymes named, and it is most probable that each mineral deficiency affects the activity of many others, but the results are of interest in suggesting a mechanism by which deficiency of a mineral element may retard protein synthesis and slow down the rate of growth, or lead to abnormal metabolism. It is possible that protein synthesis is more readily retarded, in periods of environmental duress, by a lack of one element than of another. If this is assumed to be so, and if copper, zinc, cobalt and boron are considered to have been among the most important in the circumstances of the experiment, the observed data can be accommodated.

At the opposite extreme, the observations on toxic effects of elements applied in excess were incomplete in that only two of the treatments (both involving boron) were sufficiently severe to kill the tree. It is, perhaps, permissible to assume that retarded synthesis, or breakdown of tissue, associated with highly toxic concentrations of a mineral within the plant is favourable to the establishment and growth of mealybugs. On the limited data available, however, such an assumption must be limited by the reservation that it may not hold good where the element present in excess is one to which enzymes in the insect are highly sensitive. It is conceivable that in such a case

mealybug development would also be retarded in some measure.

In the light of the foregoing data, the general conclusion may be drawn that in the conditions studied mealybug development was not prevented by lack of carbohydrate even at a light intensity as low as 15% of full daylight, and that the nitrogenous constituents of the food are of great importance in mealybug growth. If it is assumed that the quantity of nitrogenous compounds of most concern to the insect is determined by the metabolic condition of the plant, and is lowest when synthesis of protein is taking place most rapidly, and is highest when protein synthesis is retarded, or when tissues are breaking down, it is possible to explain the relationship between mealybug incidence on the host and the effects of adverse growth-conditions on the host as being determined by the effect of these conditions on the utilisation of nitrogen in the plant.

Summary

The distribution of a population of mealybugs consisting largely of *Planococcus citri* was followed for two years in plots where cacao trees, comprising three clones, were grown under different light intensities and were given different fertiliser treatments. It was found that in this environment the heaviest mealybug incidence was negatively correlated with light intensity, positively correlated with the application of nitrogen and negatively correlated with that of potassium, but showed no correlation with that of phosphorus. Heavy incidence was negatively correlated with vigour of the host plant, and this, in turn, was negatively correlated with depth of gravel below the soil surface in the rainfall conditions prevailing at the experimental site.

The incidence of mealybugs was also observed on rooted cacao cuttings subjected to various treatments. These included starvation of single nutrient elements, the administration of single nutrient elements in excessive amounts, overwatering, both in the presence of free lime and otherwise, and exposure to low diurnal humidity and high temperature.

As it was established that mealybugs were potentially associated with cacao plants with a high nitrogen status, it is possible to explain their response to host conditions induced by the experimental treatments as reflecting the effect of such treatments on the metabolism of the host, particularly with regard to the utilisation of nitrogen.

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A Stock-Scion Experiment with Cacao III

by

D. B. Murray and F. W. Cope

This experiment was planted in 1951 to investigate the growth and cropping of four clones—ICS 1, 45, 60 and 95—used as stock and scion in all combinations. Preliminary accounts have been given by Cope and Murray (1953), Murray and Cope (1955), dealing mainly with the girths of stock and scion ; in addition to further data on these girths five years yields are now available for examination.

The trees have been growing without shade since 1953 and receive regular applications of fertilisers, conditions under which the highest yields may be expected. Girth measurements have been taken annually both above and below the graft union and the crop is harvested monthly.

Experimental Results

Girth measurements

Table I gives the stock girths of the 16 combinations at September, 1958, when the trees had been seven years in the ground.

Table I

Mean stock girth (cm.), corrected for block differences, at end of seventh year of growth

Stocks	Scions: ICS 1	ICS 45	ICS 60	ICS 95	Means
ICS 1	42.2	36.7	48.7	38.3	41.5
ICS 45	34.3	27.0	38.3	29.7	32.3
ICS 60	36.2	40.6	41.0	35.1	38.2
ICS 95	37.0	31.7	42.7	32.9	36.1
Means	37.4	34.0	42.7	34.0	37.0

L.S.D. 5% marginal means ±1.97.
L.S.D. 5% same row or column in body of table ±4.61.
L.S.D. 5% not in same row or column ±4.40.

The mean girth of all ICS 1 stocks significantly exceeded that of any other stock. The scion which has imparted significantly the largest girths to all stocks is ICS 60. It is, therefore, not surprising to find that the combination of ICS 60 scion and ICS 1 stock gives plants which have significantly larger girths than any other combination. The least robust combination appears to be ICS 45 stock with ICS 45 scion.

The mean stock girths of the 16 combinations (grouped according to stock) at the end of each year of growth are presented graphically in Fig. 1. The range in girth shown by plants with ICS 60 as a rootstock is less than with any other stock. The slopes of the lines relating age and stock girth for ICS 60 scions, on all four stocks, are greater than those of other scions. The increase in stock girth of the combination, ICS 95 on ICS 45, during the seventh year is extremely small ; on the other hand, the combination, ICS 45 on ICS 60, appears to be maintaining a considerable rate of girth increase. Contrasts such as these may reflect differences in stock-scion compatibility.

Data are not separately presented for scion girths. Table II shows, instead, the mean difference in girth above and below the graft union at the end of the seventh year of growth. Positive values indicate normal tapered growth ; negative values denote an overgrowth of the stock by the scion.

Table II

Mean differences (cm.) between stock and scion girths at end of seventh year of growth

Stocks	Scions: ICS 1	ICS 45	ICS 60	ICS 95	Means
ICS 1	1.1	6.1	4.0	4.3	3.9
ICS 45	—3.2	1.8	1.1	0.5	—0.1
ICS 60	—1.1	3.4	0.4	2.7	1.3
ICS 95	0.9	0.5	0.3	1.1	0.7
Means	—0.6	2.9	1.4	2.0	1.5

If disparity of stock and scion girths is taken as giving some indication of incompatibility between stock and scion, then ICS 1, as a stock, shows the greatest degree of incompatibility with ICS 45, ICS 60 and ICS 95. ICS 1, when used as a scion on stocks of ICS 45, shows the greatest overgrowth.

Fig. 2 shows the change in stock-scion girth differences in relation to time. Stock ICS 95 seems to show less contrasted differences than other stocks.

Yields

The trees came into bearing in their third year in the field and records are available for five years cropping. The mean production over five years is given in Table III and annual yields in Fig. 3.

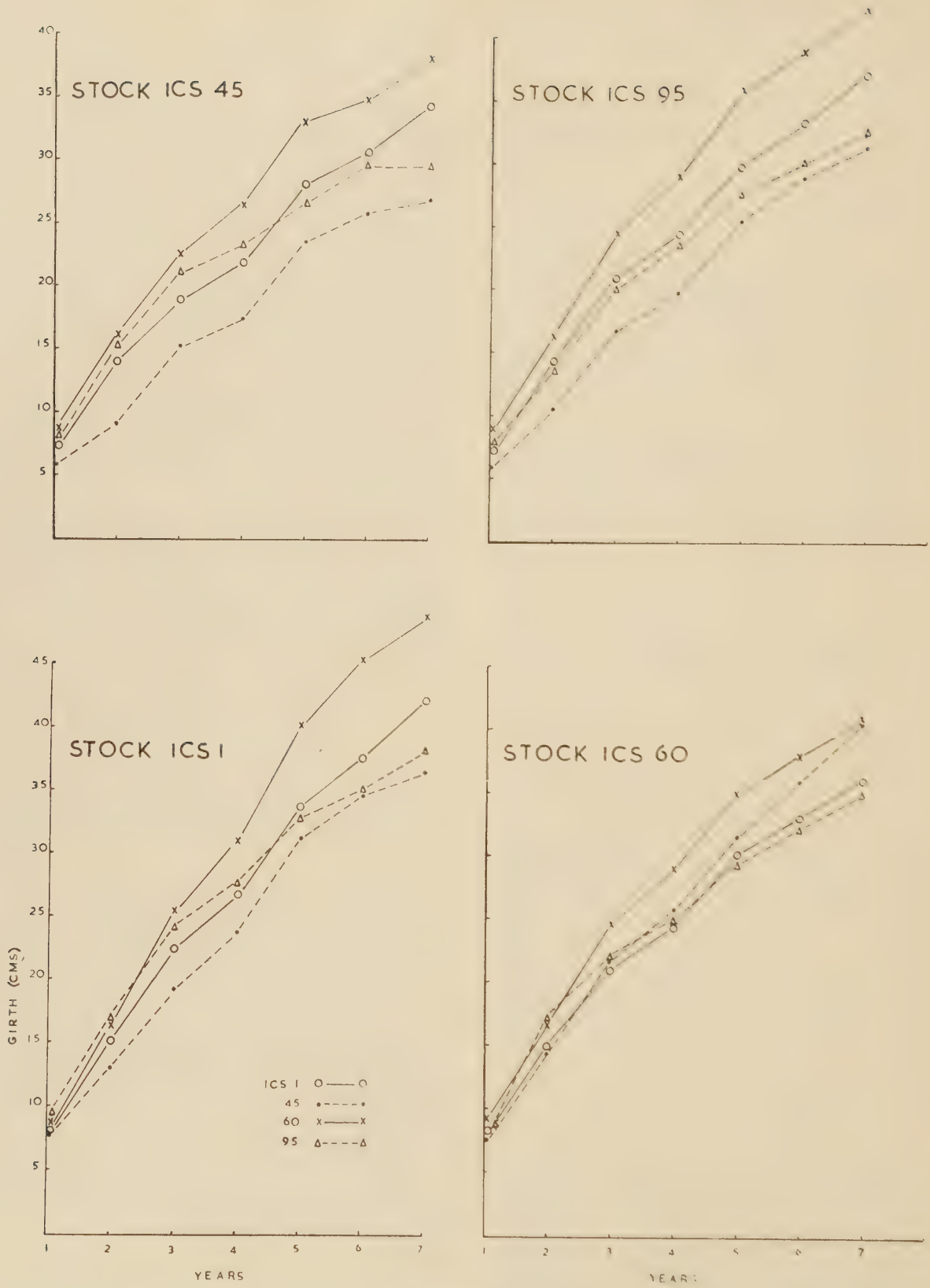
STOCK ICS 45
STOCK ICS 95
STOCK ICS 1
STOCK ICS 60
GIRTH (CMS)
YEARS
ICS 1
45
60
95

FIGURE 1

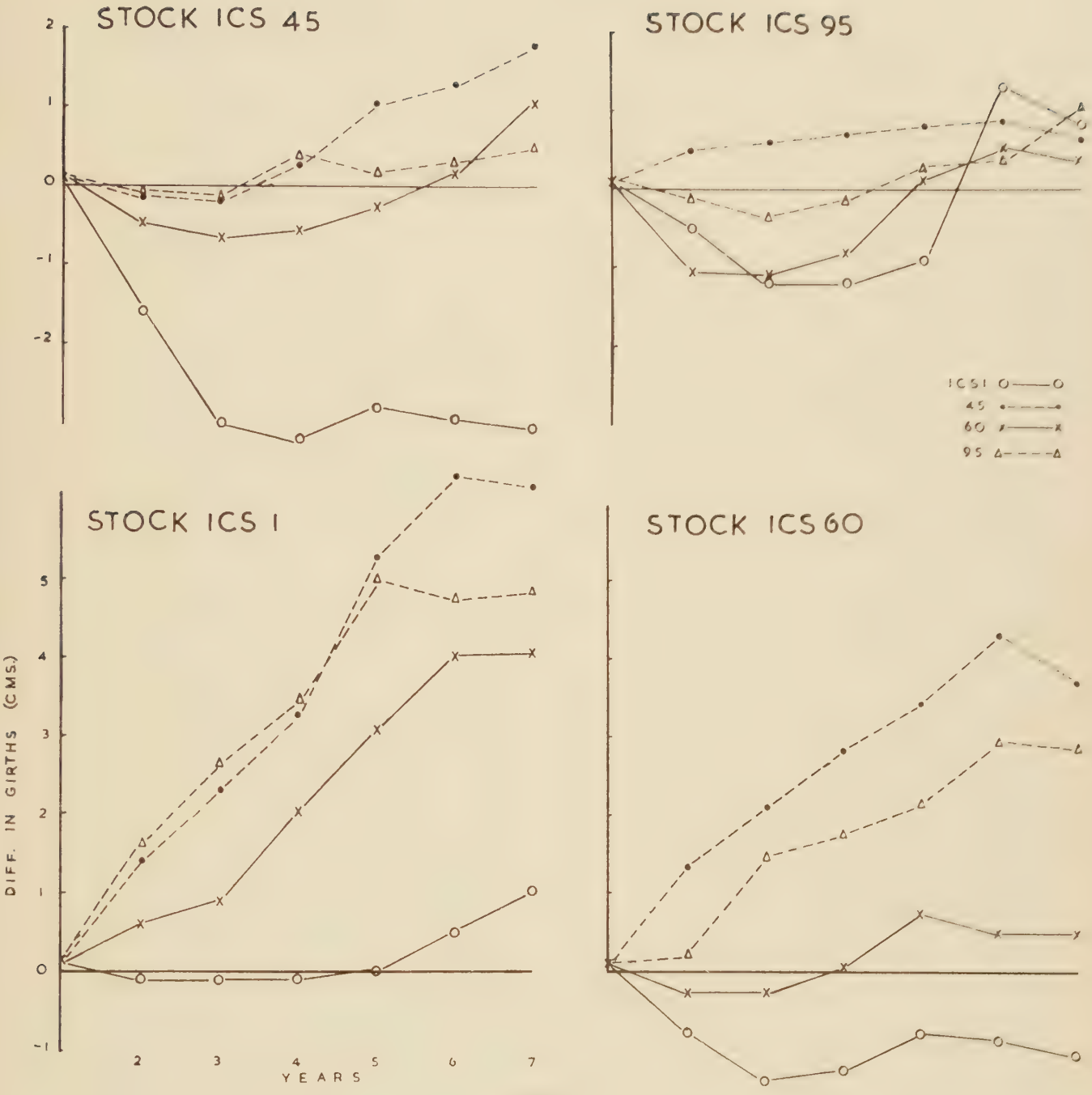
STOCK ICS 45
STOCK ICS 95
STOCK ICS 1
STOCK ICS 60
ICS1
45
60
95
DIFF. IN GIRTHS (CMS.)
YEARS
2
3
4
5
6
7

FIGURE 2

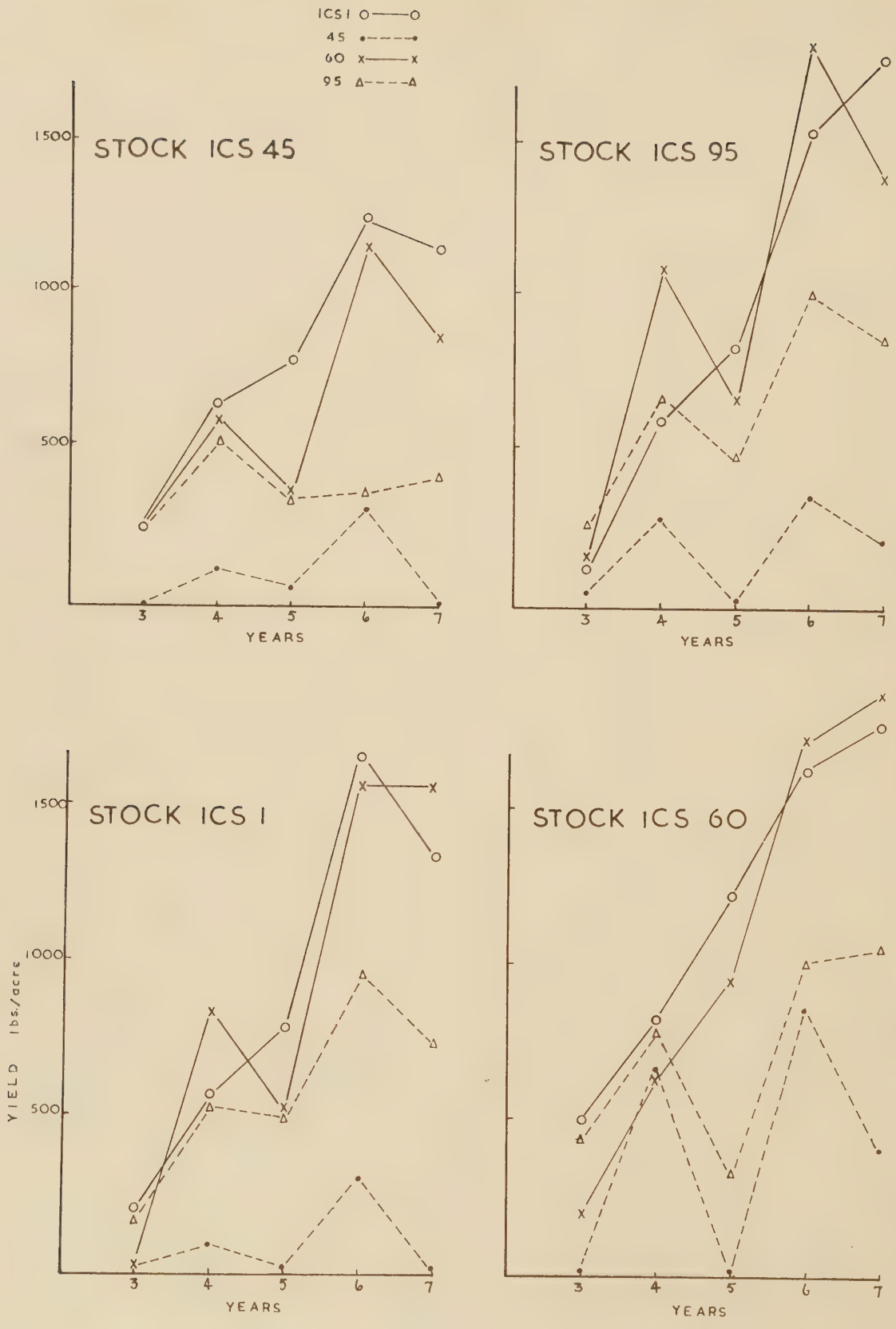
ICS 1
45
60
95
STOCK ICS 45
STOCK ICS 95
STOCK ICS 1
STOCK ICS 60
YIELD lbs./acre
1500
1000
500
3
4
5
6
7
YEARS

FIGURE 3

TABLE III

Average yield in lbs. dry cocoa/acre for 5 years, adjusted for block differences

Stocks	Scions: ICS 1	ICS 45	ICS 60	ICS 95	Means
ICS 1	1,005	81	1,000	647	683
ICS 45	891	86	720	423	530
ICS 60	1,313	431	1,197	778	930
ICS 95	1,080	212	1,132	726	787
Means	1,072	202	1,012	644	732

L.S.D. 5% marginal means ±95.

L.S.D. 5% same row or column ±222.

L.S.D. 5% not in same row or column ±212.

The analysis of variance shows stock and scion contrasts to be highly significant (P=.001). Their interactions are, however, non-significant; the differences in yield, then, between two scions (or stocks) are likely to be fairly similar with all stocks (or scions).

The annual variation in yield is highly significant and reflects, of course, both seasonal effects and the normal increase in yield of young trees. The linear regression of yield on age for both stocks and scions is very highly significant (P=.001). Higher order regressions fail to reach significance.

As regards the comparison between clones, the best stock has proved to be ICS 60; and the worst, ICS 45. As scions, however, there is no significant difference between ICS 1 and 60; ICS 45 is also the poorest as a scion.

With ICS 45 used as a stock, yields of the other three clones are all less than those realised from these clones on their own roots. This is clear confirmation of the policy adopted in propagating high-yielding selections by rooted cuttings rather than by buddings. Quite clearly a poor rootstock reduces very considerably the potential yield of a good scion.

An examination of the effects of grafting on poor scions shows that ICS 45 can give higher yields on rootstocks other than its own, but the results are not consistent. Yields of ICS 45 on ICS 1 are as low as for ICS 45 on its own roots. ICS 1 as a stock for ICS 60 or 95 gives yields as good as either on its own roots.

The best combination for yield is ICS 1 on ICS 60 which has proved better than ICS 1 on its own roots, but just not significantly better than ICS 60 on its own roots—the second highest yielder. The order of increase in yield is, however, scarcely large enough to make worthwhile the production of such double plants with clonal stocks and scions. Moreover, it would appear necessary to test experimentally all potential stocks with all the selected scions, a long and impractical procedure.

In the previous paper (Murray and Cope, 1955) an examination of girths was made along lines suggested by Vyvyan and Maggs (1954) to determine whether growth rates of combinations of clones were related to the individual components or were independent. This involves comparison between means of like unions, i.e. 1/1 and 60/60 and the corresponding unlike unions, i.e. 1/60 and 60/1. (Comparisons of this type are, of course, components of stock-scion interactions, already noted as being non-significant).

These comparisons for the total yield to date follow in Table IV.

TABLE IV

Comparison	Like mean	Unlike mean	Difference
1 and 45	546	486	60
1 and 60	1,101	1,155	54
1 and 95	865	863	2
45 and 60	641	575	66
45 and 95	406	317	89
60 and 95	961	955	6

Since these differences are small and within the limits of experimental error it follows that the yields of the combinations are directly related to the scions involved. This may also be deduced from the absence of any interaction between stock and scion in the analysis of variance. The practical conclusion can be drawn that the yield of a high yielding clone cannot be increased by growing it on a stock which is itself no higher yielding than the first clone.

TABLE V

Comparison	Yield difference		Difference due to stocks
1 and 45	$\frac{1}{60} - \frac{45}{60}$	882	
	$\frac{1}{95} - \frac{45}{95}$	868	14
1 and 60	$\frac{1}{45} - \frac{60}{45}$	171	
	$\frac{1}{95} - \frac{60}{95}$	−52	223
1 and 95	$\frac{1}{45} - \frac{95}{45}$	468	
	$\frac{1}{60} - \frac{95}{60}$	535	67
45 and 60	$\frac{60}{1} - \frac{45}{1}$	919	
	$\frac{60}{95} - \frac{45}{95}$	920	1
45 and 95	$\frac{95}{1} - \frac{45}{1}$	566	
	$\frac{95}{60} - \frac{45}{60}$	347	219
60 and 95	$\frac{60}{1} - \frac{95}{1}$	353	
	$\frac{60}{45} - \frac{95}{45}$	297	56

A further type of comparison is of interest. It involves the question—is the difference in yield of two clones when used as scions the same irrespective of rootstocks? For example, is the difference in yield between 1/45 and 60/45

the same as that between 1/95 and 60/95? These comparisons are made in Table V.

It would appear that for four of the comparisons agreement is very good, i.e. the differences in yield of scions is the same irrespective of stock. The agreement is poorer for the comparisons of 1 and 95 and 45 and 95, but the differences would be within the range of experimental error. The conclusion may be drawn that the yields of two scions are each affected to the same relative extent by the stock on which they are grown.

The comparison of the clones as stocks gives similar results and the figures are not reproduced.

Of some interest is the different pattern of cropping shown by ICS 1 as a scion when compared with the other scions. In all cases involving ICS 1 as a scion yields increased from the fourth to the fifth year. All the remaining 12 combinations showed a drop in yield in the same period with the exception of ICS 60 on ICS 60. The general fall in yield may be attributed to a poor season, short in rainfall, but it would appear unusual that one scion should react so differently from the others, irrespective of the rootstock on which it is growing.

An explanation of this effect is not easy to arrive at. One possibility might be that ICS 1 is more tolerant to dry conditions through a lower foliar water loss in the dry season. There is little evidence, either experimental or observational, that ICS 1 is more drought resistant than the other clones, but this may be the case. The fact remains that it is not a soil factor that is responsible, but some factor in the aerial environment to which ICS 1 reacts differently from the other clones.

When the monthly cropping pattern is examined the difference shows up clearly. Fig. 4 shows the number of pods harvested per month from the two combinations, 1/60 and 60/1, which have produced much the same total crop over the five-year period. The overall pattern is that of young cacao coming into bearing; Murray (1955) has shown that little crop is harvested in the December January period, nearly all coming in the late crop period of May/June. With increasing age there is a reversal of phase so that the majority of the crop ripens in the main crop period (December/January) and a smaller peak occurs in May/June. This periodicity must be determined mainly by the environmental factor of rainfall, but the interesting point is that the two combinations have reacted so differently, particularly in the fifth and sixth years of cropping. In both these years 1/60 has given peak crops in the late dry season when 60/1 has given only a small crop at the same time. The pattern of cropping of 1/60 follows that of 1/1 (not reproduced) and 60/1 follows 60/60. It would therefore appear that the cropping pattern is determined more markedly by the scion than by the stock. As stated earlier, this must mean that the different scions react differently to the aerial environment.

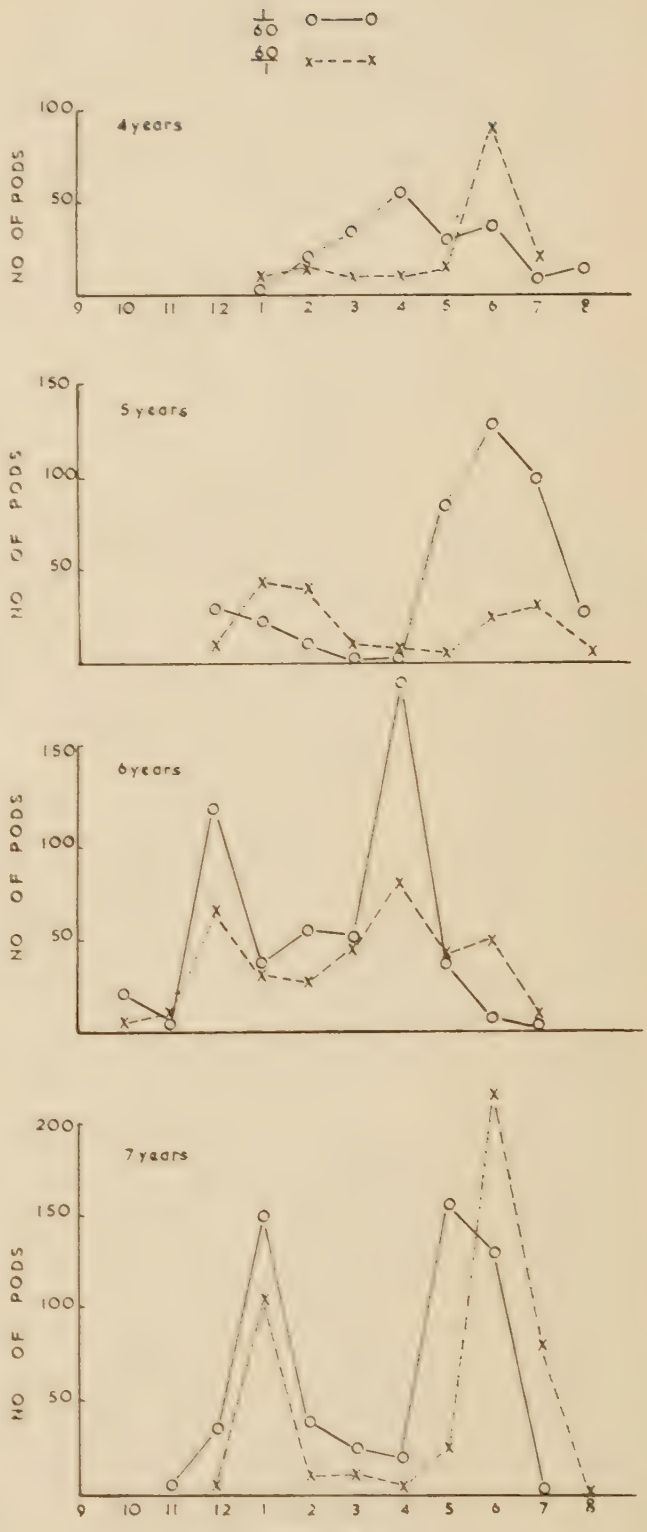

FIGURE 4

Girth and yield

Jones and Maliphant (1958) have demonstrated a highly significant correlation between girth at 3½ years and yield in the sixth to eighth years. No such correlation exists in these double plants. There is some indication that the lowest yields are associated with the smallest girths, but among the high-yielding combinations yield bear little relation to girth. Jones and Maliphant, however, were concerned only with a single clone. Moreover, there is the added factor here of the graft union and the differential growth of stock and scion.

Weight of beans per pod

The weight of beans per pod in cacao is determined genetically, but may be modified to some extent by nutrition and more especially water availability, that is, pods tend to give smaller weights of beans when they develop through the dry season as compared with development in the wet season.

The average weight of beans per pod for the 1956-57 season is given in Table VI.

TABLE VI

Mean weight of wet beans per pod in gms.

		Scions				
		ICS 1	ICS 45	ICS 60	ICS 95	Means
Stocks	ICS 1	120	79	155	94	118
	ICS 45	120	105	158	67	117
	ICS 60	130	113	168	93	128
	ICS 95	126	99	142	92	119
	Means	124	102	155	89	121

The figures reflect a considerable amount of variation and a full analysis would involve differentiation between dry season and wet season cropping, an analysis not considered worth while. The general pattern, however, shows the bean weight to be largely determined by the scion (as shown by the comparison of scion means). The similarity of the stock means indicates the relative unimportance of the stock in influencing the average weight of beans per pod. These results might be expected from the absence of any interaction between stock and scion already demonstrated.

Discussion

From the practical point of view the results of this experiment serve to confirm early policy in using selected material as cuttings on their own roots rather than as buddings on unknown seedling rootstocks. There is, it would appear, no prospect of producing higher yields with particular combinations than with single clone plants, as it has been shown that scion and stock do not interact. The combination of two comparable high yielding clones does not give a yield any higher than that obtained from each clone individually. It would be possible, however, to improve the yield of a weak criollo clone (e.g. ICS 45) by growing it on a more vigorous stock.

The possibility of using a dwarfing stock to make cultivation and harvesting easier is also precluded. The stock with the greatest dwarfing effect, ICS 45, gives poor yields even with a potentially high yielding scion. The yields on a mutant dwarf, not included in this experiment, have also been extremely poor. High yields are associated with vigorous plants even though the girth correlation is not significant.

Should it be desired to grow a particular flavour type, which is low yielding on its own roots, yield could be improved by a stock such as ICS 60. A question not yet answered is, will a Witches' broom resistant clone (such as SCA 6) impart any resistance to a susceptible scion? In view of the negligible interaction between stock and scion this would appear unlikely, but the matter is under trial in another stock-scion experiment planted at River Estate.

Summary

(1) A stock-scion experiment with cacao is described in which four clones have been combined as scion and rootstock in all 16 combinations.

(2) Differences in growth as measured by girth of stock and scion are analysed.

(3) With respect to yield, a poor clone will give a higher yield on a good stock and the converse also holds that yields of a good scion are reduced by a poor stock. This provides confirmation of the early policy (in vegetative propagation) of using cuttings on their own roots rather than buddings on unproven seedling stocks. No combination gave significantly higher yields than the best clone on its own roots.

(4) The interaction between stock and scion is negligible. It follows that differences between yields of scions are the same irrespective of rootstock.

(5) The cropping pattern of one clone as scion differs markedly from the others and would seem to indicate a differential response to the aerial environment.

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Las Hermanas — the new sub-station

by

D. B. Murray

WHEN the Cocoa Research Scheme was initiated in 1930 the amount of land suitable for experimental purposes at the College itself was extremely limited. As a result, field experiments were mainly carried out with the co-operation of the Trinidad Department of Agriculture at their River Estate Research Station. This situation continued up to 1947 when, as a result of negotiations the details of which will not be considered here, River Estate was leased to the College as the field centre for cacao research. From that date a large number of field trials have been laid down there, the results of which are gradually becoming available.

Cacao, however, is a crop which is very sensitive to its environment and the results secured from a single experimental site cannot always be related directly to other sites. This point is well illustrated by the clone ICS 1 which gave outstanding yields at River Estate in the early CRB trials ; as a result of its performance there, it was propagated in large numbers by the Cocoa Board of Trinidad and Tobago for distribution to estates. Its performance on heavy clay soils with impeded drainage was, however, very disappointing, yields seldom reaching even half those achieved at River Estate.

As a purely local problem, this matter has been tackled by the Cocoa Board, which has laid down a series of trials of the various clones on a wide range of soil types. These will serve to indicate which clones give the best performance in the various areas and distribution to estates will be arranged accordingly.

As distinct from the local problem, however, there remained the more fundamental questions involved in the growth of cacao on heavy soils. This is a matter not only of the suitability of any clone or clones, but of the wider fields of general growth and cropping, responses to fertilisers and shade, water relations, etc.

At one time it was hoped to duplicate some of the River Estate experiments on the land of the Central Experiment Station of the Department of Agriculture at Centeno, but this plan never materialised. Attempts were, therefore, made to secure suitable experimental facilities on existing cacao estates in co-operation with the owners.

A number of estates were visited by one or more members of the Cocoa Research Scheme, but for various reasons, distance from the College, unsuitable topography, etc., no suitable area was found until 1955 when the Las Hermanas Estate changed hands and the new owners indicated their willingness to release part of the estate for experimental purposes.

Las Hermanas is situated on the Tumpuna River some 15 miles by road east of the College. This ensures that the minimum of time is spent in travelling, an important consideration. (River Estate by comparison is some 18 miles west of St. Augustine.) The soil type is the Cunupia clay loam to silty clay, a type representative of a large area of land on which cacao has been grown in the past, but on which it is proving difficult to replant and secure good yields. It is an acid soil, pH 4.5 to 6.5, low in phosphate, potash and calcium and the profile shows the typical indications of impeded drainage and consequent inadequate aeration.

Negotiations over the 25-year lease of 100 acres of the estate were rather protracted so that it was not until May, 1957, that work could start on the site. By then the rainy season was about to begin and it was too late to prepare any land for planting that year.

The first necessity was to clear an area near the access road and build an office and storerooms, a cottage for a resident labourer-watchman and a shed with a glass roof for storage of plants. These buildings were erected by a local contractor. The site has no mains water or electricity so that a portable pump is used to pump water from the Tumpuna river in addition to rain water collected from roofs and stored in tanks.

Field operations began in earnest with the dry season of early 1958. Choice of experimental sites has not been easy owing to the existence of large clumps of bamboos among the old cacao and occasional very deep drains. Areas were cleared for a large progeny trial, a shade and fertiliser experiment and a soil cultivation trial. Clearing was done by hand except for the last trial where a Caterpillar D4 bulldozer pushed over and wind-rowed some 8 acres of the old cacao and immortelles ; a Cuthbertson draining plough was used for the preparation of the drains. Several hundred yards of rough roads have also been made and surfaced with gravel.

With the 1958 rains the progeny trial and the shade experiment have been planted. The soil cultivation trial will not be planted until 1959, only the banana shade being planted in 1958.

The period of development has not been without incident. The overseer travelling one pay day on the rather deserted access road during a heavy rainstorm had a rock hurled through his windscreen in an attempted pay roll robbery. Luckily he was not hurt and did not

stop ; but he now travels with an armed watchman. Then, after a period of very heavy rain the Tumpuna river, which in the dry season is some 25 ft. below the wooden bridge leading to the office area, rose to cover the bridge to the height of the lower handrail. Fortunately, it subsided before any damage was done to the bridge.

Some idea of the run-down nature of the old cacao may be gathered from the fact that during the 1957-58 crop some 30 acres of land gave 4,000 lb. dry cacao. A yield of 130 lb./acre is quite uneconomic and it is confidently expected that the experimental work now being undertaken will eventually show how to secure yields several times this figure on these difficult types of soil.

FIGURE 1
Approach over the Tumpuna River.

FIGURE 2
Land clearing with Caterpillar D4 bulldozer.

FIGURE 3
Office and storeroom, watchman's cottage behind.

FIGURE 4
Shade experiment.

Propagation of Cacao in Plastic Bags*

by

R. Nichols

THE basic physiological requirements for the vegetative propagation of cacao (*Theobroma cacao*) have been broadly worked out by Evans (1951) and may be summarised in that semi-hardwood cuttings in an atmosphere near 100% relative humidity, at a reduced light intensity (15-20% of the incident sunlight), given adequate aeration at the base of the cutting, will produce roots in the order of 70-90% rooting efficiency.

A comparison of the costs of commercial propagation in Trinidad has been made by Murray and Bridge (1956), and McKelvie (1957) has shown that cacao may be propagated at greatly reduced cost, on a commercial scale, using sawdust-cored baskets (Murray, 1954) containing cacao cuttings, covered by polyethylene sheets. This method adequately dispenses with the expensive installation of commercial propagating bins, and considerably reduces water consumption and labour costs. Another method, making use of the properties of polyethylene, which restricts water-vapour diffusion, but permits some gaseous diffusion, is being developed here. Cuttings are rooted entirely in plastic bags, transported to the field in the same fashion and afterwards established in the field.

The base of a semi-hardwood cacao cutting bearing 4-6 trimmed leaves is dipped in hormone solution. The base of the cutting is first placed in wet, leached sawdust laid in coconut fibre. The fibre is then folded around the base of the cutting and held in place with an elastic band. The final size of the sawdust and fibre " ball " is about the size of a half-clenched fist. The cutting is inserted into a plastic bag (150 gauge, 20 in.×11 in.), water (150-200 ml.) added and the neck closed with string. The bag is suspended under a shade of domestic cloth, and no further attention is required until root formation has occurred. The roots emerge through the fibre and may be readily seen through the plastic. The light-intensity, calculated by means of an actinograph, is approximately 7% of the incident sunlight, but is not critical over wide limits providing the light is diffused.

The cutting is transported to the field in the bag and planted under heavy shade with the roots still enclosed in the fibre. The surplus water from the plastic bag is tipped over the plant and the bag is inverted over the cutting with the mouth of the bag lying loosely upon the soil surface. Two banana leaves, which provide local shade, are arranged in the form of an inverted-V over the cutting, with the petioles firmly pressed into the soil. Natural drying-out of the banana leaves permits progressively more light to fall on the cutting. After 4-5 days, the bag is removed and re-used for further propagation. The purpose of the inverted bag is to reduce transpiration and replaces the conventional hardening procedure. The probable reason why success is achieved by this method is that the roots of the cutting are intimately interwoven with the wet fibre and sawdust, which retains moisture for a considerable time during the critical period when the roots are becoming established in the soil.

The rooting percentage in the bag is of the order of 80-90, depending upon the clone, and establishment in the field about 60-70% of the cuttings set in the bags. Plants of the more vigorous clones are ready for planting 22 days from setting.

The advantages of the method are many. The plastic bag costs 2½d. and may be used a number of times without deterioration. Labour charges and initial installation are negligible when compared with conventional methods. Plants are introduced into the field more quickly and are very easily transported by hanging the bags from wires in a vehicle. Furthermore, drying-out, which is frequently observed with plants in baskets transported over long distances, does not occur. The combined weight of a plant and basket is between 3½ and 4 lb. as compared with 1 lb. for a plant in a plastic bag. One man may carry up to 20 plants in bags without difficulty, whereas he is restricted to two basketed plants owing to the weight and shape. This means that inaccessible bush areas may be more readily planted with clonal cacao. Skilled labour is not required at any stage in the process, and normal care is all that is required at planting.

This method will not replace conventional commercial procedure owing to the difficulties of storage. It is of considerable value, however, to the planter who wishes to propagate plants in order to replace dead plants or to plant new areas, without having to await supplies from a central propagating station.

The method has proved of considerable value at River Estate where, owing to the presence of mild virus strains, propagation must be done at site rather than by sending material to the college propagating unit at St. Augustine. The technique has been modified there to accommodate the problem of storage. After rooting in the bags, the plants are taken out with the roots still in the ball of fibre and inserted into an old potting basket partly filled with soil ; more soil is added to fill the basket. The plant is then transferred to a shaded greenhouse floor without further hardening or attention, except for the usual watering regime. In this way 66% of the cuttings set have been established, a high figure considering that the cuttings are taken from the field plantings of ICS × Scavina hybrids and not from nursery trees.

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Summary

An inexpensive method for the propagation of cacao in polyethylene bags is described.

Acknowledgments

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The effect of gibberellic acid on cacao seedlings

by

R. Nichols

A RICE disease caused by the fungus *Gibberella fujikuroi* first stimulated the interest of Japanese pathologists some 30 years ago and has resulted in the initiation of the now copious literature on gibberellic acid. The curious syndrome of the disease by which infected plants were taller and thinner, but flowered and died earlier than their healthy counterparts led to the name " foolish seedling " or bakanae disease. Ultimately two chemical by-products of the fungal metabolism were found to be responsible for the behaviour of the infected plants. One, the toxic factor was shown to be fusarinic acid, the second which accelerated growth was shown to be a compound or complex to which the general name gibberellin was given. By large-scale fermentation using the imperfect stage of the fungus *Fusarium moniliforme*, a compound named by British workers gibberellic acid (GA) has become available for experimental purposes. GA has been shown to have similar physiological properties to the active compound gibberellin A of the Japanese workers.

It was of considerable interest to examine the effects of GA on cacao because some of its reported effects on other plants, e.g. increase in growth rate, increase in dry weight, breaking of apical dominance, induction of early flowering, etc. (Stowe and Yamaki 1957) might be of importance in cacao.

Materials and Methods

Seeds from open-pollinated ICS 95 pods were germinated and the young plants were transferred to 8 in. flower pots containing ordinary potting soil. After 8 weeks the plants were selected for uniformity and placed in an insect-proof greenhouse. The only treatments other than those recorded in the following sections were twice daily waterings and occasional treatments with parathion to control red spider.

GA was applied as an aqueous spray containing a few drops of " Teepol " as a spreader using a " Dronwal " mist-sprayer. Plants were treated individually and sprayed on upper and lower leaf-surfaces. In experiments involving nutrient sprays the relevant salts were dissolved in the GA solutions.

Results

In the first experiment GA was sprayed at fortnightly intervals at concentrations of 0, 1, 10 and 100 p.p.m. GA. At 3 weeks the plants were showing signs of nutrient deficiencies and, therefore, a single application of 0.5% urea and 0.5% KH_2PO_4 was applied as a foliar spray. After 10 days the plants receiving the 100 p.p.m. spray had shown a significant increase in height and after 5 weeks these plants were almost double the height of the water-treated controls. The increase in height with time, expressed as the mean percentage increase of the original height, is shown in Fig. 1. At 1 p.p.m. GA is ineffective whereas at 10 p.p.m. a significant increase in growth occurs. All the increase in growth is by internode elongation, the plants receiving the highest concentration showing small leaves with elongated petioles and a delicate main stem. The leaves were very pale yellow showing apparently severe chlorosis and nitrogen deficiency ; the plants had the general appearance of being severely etiolated.

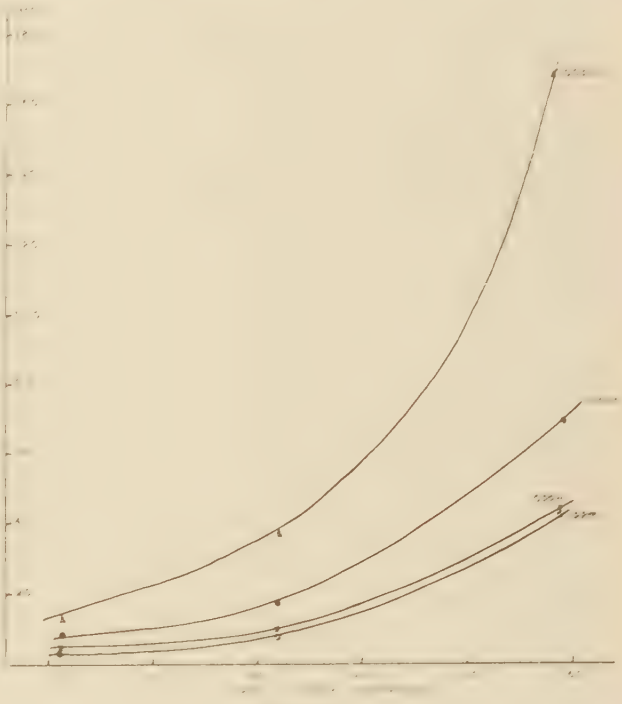

FIGURE 1
Effect of GA on cacao seedlings.

At 4 weeks from the commencement of treatment, 4 plants from the 0, 10 and 100 p.p.m. treatments were analysed for total nitrogen and the dry and fresh weights determined—see Table I.

The roots show little change in dry and fresh weights as a result of the GA treatment. At 10 p.p.m., GA gives a significant increase in the dry weight of the shoots, but at 100 p.p.m. the apparent increase in dry weight just fails to be significant. The apparent increase in fresh weight of the shoots was not significant. Nitrogen expressed as a percentage of the dry weight remains constant.

TABLE I

Concentration of GA p.p.m.	Fresh weight gms.		Dry weight gms.		%N	
	Roots	Shoots	Roots	Shoots	Roots	Shoots
0	2.1	11.9	0.28	3.0	1.6	2.45
10	2.2	15.4	0.29	3.98*	1.53	2.28
100	2.05	15.7	0.26	3.90	1.66	2.26

* Significant increase at 5% level.

The disturbing feature about this experiment was the apparent inability of the treated plants to take up sufficient nutrients to match the phenomenal increase in growth rate of the shoots. Subsequently a similar experiment was designed to examine the effects of foliar nutrient sprays as a means of supplying sufficient nutrient to satisfy the increased demand by the plant. The treatments involved spraying GA (50 p.p.m.) with and without foliar nutrient sprays at weekly and monthly intervals. There were five treatments :—

(1) Control—water spray only and GA.

(2) Spray—plus nutrient weekly, minus GA.

(3) ,, —minus nutrient, plus GA weekly.

(4) ,, —plus nutrient weekly, plus GA weekly.

(5) ,, —plus nutrient weekly, plus GA monthly.

GA at 50 p.p.m. was chosen as a concentration which from the previous experiment would induce a considerable growth effect. The nutrient spray was 0.75% urea + 0.75% KH_2PO_4 applied weekly. Ferrosene (iron chelate) 0.5 gms. was applied to the soil of each pot as a dry powder at three weekly intervals.

The results were not appreciably different from the first experiment except that the control plants over the 3 month period of observation remained perfectly healthy and the deficiency symptoms of the GA treated plants were much reduced in treatments 4 and 5. However, the same spindly habit appeared particularly in treatments 3 and 4. It was clearly obvious that the GA treated plants would not stand up to field conditions owing to the weakness of the main stem, frequently accompanied by longitudinal splits in the epidermis caused by overgrowth of the secondary stem tissues.

Several interesting features, however, arose from the plants sprayed at weekly intervals. It has been reported that GA sprayed on to other plants is not toxic at even relatively high physiological concentrations (>1,000 p.p.m.) but in these experiments the death of the terminal bud occurred in half the plants treated with 50 p.p.m. GA weekly. It appeared that the normal "flushing" cycle of the seedling was disturbed in that the short near-dormant period between each "flush" of the apical meristem did not occur. The GA in fact, induced almost continuous growth. Under these circumstances the terminal bud and meristem died. As soon as the bud died apical dominance was lost, but instead of an axillary bud nearest the apex sprouting as is the case with a decapitated cacao seedling, three or four axillary buds of the GA treated plants sprouted at the same time. Below the necrotic terminal bud a typical swelling of the stem appeared, about ½ in. in length not unlike the swollen cotyledonary node of a seedling infected with Witches' broom (*Marasmius perniciosus* Stahel). The bushy type habit of the seedling persisted for as long as the observations were carried on (4½ months) without any indication that one or other branch would assume dominance. The interesting feature is that whilst in some plants which have been treated with GA loss of apical dominance is commonly observed, with cacao it is only after treatment with GA and eventual death of the terminal bud that apical dominance is lost.

Application of GA in lanolin to seedlings

A 1.25% paste of GA in lanolin was prepared by dissolving 0.0125 gms. of GA in 1 gm. of warm lanolin to which 7 drops of teepol had been added (Marth *et al.* 1956). Light smears of the paste were applied just below the apical buds of 10 cacao seedlings.

The seedlings responded to the GA more slowly than aqueous sprays in spite of the higher concentration, but the result was similar (Fig. 2).

FIGURE 2

Effect of GA applied as a lanolin emulsion. Controls on left, 1.25% GA on right.

Effect on cuttings

GA sprayed on 10 well rooted cuttings at 50 p.p.m. failed to show any response.

When it became clear that GA was unlikely to have any useful commercial effects on cacao further detailed observations and spray treatments were concluded. It is of interest to note that once spraying has ceased the GA treated plants "grow away" from the apparent etiolation effect and resume normal growth (Fig. 3).

Discussion

Although cacao seedlings respond to GA by an accelerated growth rate from the experimental evidence there appears to be little prospect for its use commercially as a growth stimulant. The increase in growth observed is mainly due to marked elongation of the internodes and at 100 p.p.m. the young leaves are much reduced in size. The plant as a whole cannot maintain an adequate nutrient balance, but perhaps the most obvious effect is the very weak stem which results from the treatment and, therefore, at the best, on seedlings at least, GA sprays can be only of academic interest.

FIGURE 3

Left to *right*, 0, 10, 100 p.p.m.—after treatment had ceased. The treated plants are recovering and resuming normal growth.

However the effect of breaking apical dominance, the accelerated growth and the swelling of the stem suggested certain similarities with the symptoms produced on cacao by Witches' broom fungus and led to the work reported elsewhere, Dudman and Nichols (1959). The implication was that the Witches' broom fungus may produce gibberellin-like compounds *in vivo*, GA itself being a by-product of the metabolism of the fungus *Fusarium moniliforme*.

Acknowledgments

Grateful thanks are due to Dr. P. W. Brian of I.C.I. for samples of gibberellic acid and to Merck Chemical Co. for supplies of " Gibrel," a potassium salt of gibberellic acid.

Summary

(1) The effects of gibberellic acid at various concentrations on cacao seedlings are reported.

(2) Gibberellic acid has apparently no effect on cuttings.

(3) Owing to the weakened nature of the plant after treatment, the accelerated growth rate which is the main effect, is of little value.

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The Growth Substances of Theobroma cacao II

by

R. Nichols

Introduction

In a previous communication (Nichols 1957) it was shown by extraction of large quantities of cacao tissue that three compounds active in the wheat coleoptile straight growth test could be detected. They were named cacao auxin I, cacao auxin II and the acidic inhibitor. Cacao auxin I, a growth accelerator was shown to be an acid similar in Rf behaviour to indolyl acetic acid (IAA) but not identical to it. Cacao auxin II was shown to be neutral in reaction and was characterised solely by its Rf behaviour. The acidic inhibitor, probably a complex of compounds present in most cacao tissues examined, is almost certainly of wide distribution in other plant tissues.

This communication describes the quantitative examination of cherelles by the oat curvature test. This test is very sensitive to low concentrations of auxin permitting investigation of small quantities of tissue ; the test has the further value that only substances translocated in agar are detected.

Materials and Methods

Oats v. *Victory* are soaked for 1 hour in water and germinated on damp blotting paper for 24 hours. The oats are then sown in perspex boxes containing washed beach sand well mixed with water to a final moisture content of 15% w/w. The germinated oats are selected for uniformity, transferred to an air-conditioned room maintained at 23.5°C.±1.0°C. at 90-95% humidity and grown for a further 48 hours under weak red light. All further manipulations are carried out in the air-conditioned room under red light, shown by experiment to be phototropically inactive.

When the oats are approximately 2 cms. tall, some 72 hours from soaking, the tips are removed. Three hours later, a further segment is removed from the coleoptile sheath and the primary leaf exposed by this operation is broken at the meristem and trimmed to a convenient height. Agar blocks containing synthetic auxin or extracts from plant tissue are then placed unilaterally on the shoulder of the coleoptile and 1½ hours later the curvature of the coleoptiles is recorded by means of a shadowgraph (Went and Thimann 1937 ; van Overbeek 1950).

It was found in common with other workers using this technique that there was a day to day variation in the sensitivity of the coleoptiles to known concentrations of applied auxin. It was, therefore, necessary to establish the relationship between concentration and curvature on various days ; Fig. 1 shows five such determinations. For

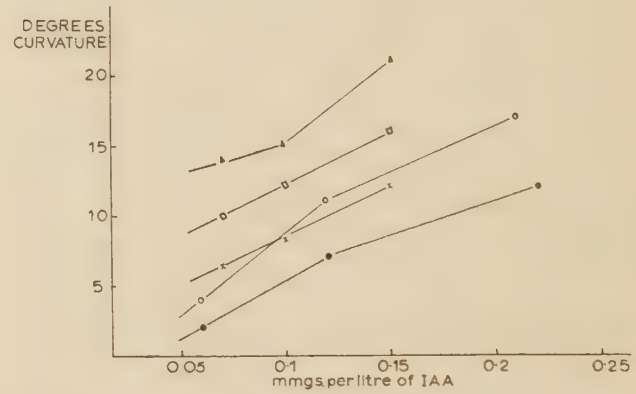

Figure 1

Relation between IAA in the agar blocks and curvature of the coleoptiles in the oat test. Data for 5 different days showing extremes of sensitivity to applied IAA.

the sake of clarity not all points are included, but it may be seen that over the concentration range for IAA, from 0.06 mgms./l. to approximately 0.2 mgms./l. there is a linear relationship between concentration and curvature. At concentrations above 0.2 mgms./l. the curves flatten out and, therefore, extracts which give curvatures above this value have to be suitably diluted. During any particular test, a control batch of coleoptiles is treated with agar containing 0.12 mgms./l. IAA in order to establish the degree of sensitivity of the coleoptiles on that particular day. From the curve corresponding to the curvature produced by the 0.12 mgms./l. IAA, the concentration of active auxin in the extracts, may be determined in IAA equivalents from the formula :—

$$\frac{F \times Va}{w},$$

where F is the conversion factor in IAA equivalents, Va is the volume of agar in ml. and w the weight of tissue in grams.

The general method of extraction used has been to freeze dry the fresh tissue, grind to a fine powder and extract with wet ether overnight at 5°C. Modifications of this procedure are described in the text where appropriate.

Results

Examination of pods

It is generally considered that the active auxin-producing centres of fruits are the ovules and, therefore, for cherelle investigations, all tissues internal or readily separable from the pod wall are extracted. Using the freeze-drying technique, approximately 60 gms. of tissue may be accommodated in the apparatus available. This limitation is reflected in the number of pods actually extracted of any particular size, i.e. as the pods develop and increase in size, fewer are required to make a given weight. Hence in Fig. 2 the results of pod extractions are expressed as IAA equivalents $\times$ 10^{-2} per unit pod. Expressing the results in this way the auxin activity may be shown to be related to the wilting index.

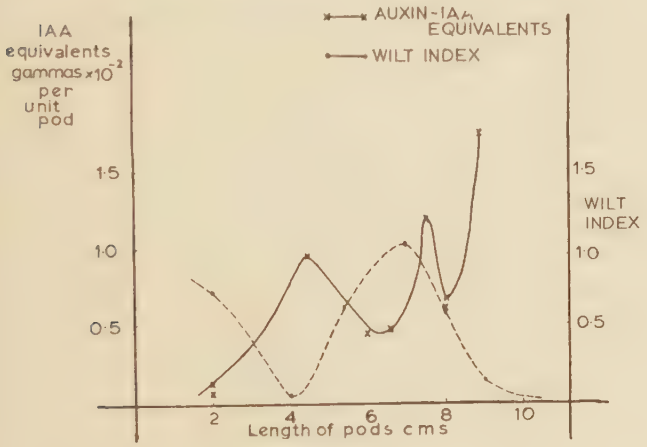

FIGURE 2

Relation between endogenous auxin and length of cherelles. The points on the curve for the wilt index have been abstracted from the data of Akinbode (1958).

Several workers have shown that the predisposition of pods to wilt is not a continuous process from fertilisation to maturity, but that at certain times in their life history there are periods at which wilt is more likely to occur than others. This observation has been correlated with internal changes in the ovules.

The most satisfactory method of expressing wilt is by the " wilt index," which has been determined in Trinidad by Akinbode (1958) working in this laboratory. Superimposed upon the auxin values recorded in Fig. 2 are figures for the wilt index obtained during 1957, determined at the same time of the year as the auxin determinations in 1958.

Akinbode found only one major peak of wilt 6-7 weeks from fertilisation. This peak followed the initial high wilt after the pollinations made during December, with a period of low wilt in between. During the early part of the wet season, from July onwards, he recorded a second wave of wilt some 9 weeks from fertilisation.

It may be clearly seen from Fig. 2 that an approximate inverse relationship is apparent between the wilt index and the auxin concentration. This result may be expected if the hypothesis is advanced that wilt is controlled by production of endogenous auxin. It does not hold quantitatively when the pods are 6-7 cms. in length because it may be seen that although the auxin level drops, it does not reach the near zero level following fertilisation, although at this time wilt index is at a maximum. However important metabolic changes are going on in the pods at this same period commencing with the division of the zygote, followed rapidly by fat synthesis, starch and sucrose accumulation, factors which must markedly affect the ultimate fate of any particular pod. How closely these metabolic changes are connected with auxin changes is not known.

The highest level of auxin activity recorded is in the pods 9-10 cms. long, approximately 10 weeks from germination. Pods older than this are rarely subject to physiological wilt, and it has been shown using a different technique (Nichols 1957) that both cacao auxins I and II are present in older pods, evidence which cumulatively supports the hypothesis of hormonal control of wilt.

Further examination of auxins I and II

It was conceivable that auxin I found by the straight growth test was not the same auxin which gave the curvature in the oat curvature technique. The curvature technique, owing to its relatively higher sensitivity, might detect auxins which would otherwise be missed in the straight growth test. This possibility was tested by running duplicate extracts of pod tissue on a chromatogram and bioassaying both in the region corresponding to the expected presence of auxin I ; in this way auxin II and the acid inhibitor are removed. The presence of auxin I was confirmed by the usual straight growth method from one extract and the corresponding Rf area of the other extract was eluted for 1 hour with wet ether. The ether extract was concentrated and evaporated over agar and subsequently bioassayed by the curvature technique. A curvature equivalent to 0.12 mgms./l. IAA was obtained, illustrating that auxin I is identical to the auxin I found by the straight growth test.

It was also necessary to determine whether auxin II was active in the curvature test. To examine this hypothesis large pods (approximately 16 cms. long) which have been shown to be a rich source of auxin II were extracted and chromatograms of the ether concentrate were run in isopropanol/ammonia. In this instance the lower third of the chromatogram was re-eluted with ether and the ether concentrate was bioassayed ; in this way auxin I and the acidic inhibitor were eliminated from the test. The result of this experiment showed that auxin II was also active in the curvature test and, therefore, the results shown in Fig. 2 are almost certainly a combined effect of the presence of both auxins.

The nature of cacao auxin I has been further examined by large scale extracts of pods 5-9 cms. in length. By comparison with other tissues examined cacao fruits appear to be low in auxin and in order to detect chemically endogenous auxin, large quantities of tissue have to be extracted. Because of its Rf values and by analogy to other plants, there is some presumptive argument that auxin I is similar to IAA. In order to examine this possibility 5 kgms. of ovule tissue taken from 1,800 cherelles 5-9 cms. in length, were deep frozen in solid carbon dioxide and alcohol (rum spirit). The tissues were allowed to return to room temperature and macerated in a Waring blender. After maceration, the alcoholic extract

was stored in a refrigerator. The process of collection of pods and maceration took 4 days. The extracts were bulked, filtered and the alcohol removed *in vacuo*. The aqueous residue was partitioned through sodium bicarbonate and ether and the final ether solution containing the acidic substances was concentrated and chromatogrammed in isopropanol ammonia.

The chromatogram was bioassayed by the straight growth technique. The Rf of IAA run as a marker was 0.31, whilst the area of the chromatogram bioassayed, which gave a significant increase in the straight growth of the coleoptiles at the 5% fiducial limits corresponded to an Rf of between 0.27 and 0.33. A duplicate example of the extract run as a chromatogram gave a transient red colour with $FeCl_3$ $HClO_4$ at a similar Rf. The evidence, therefore, points to auxin I being similar to IAA with the proviso that the transient nature of the colour reaction with $FeCl_3$ $HClO_4$ is not typical of IAA, and other compounds are known to give a similar colour reaction at the Rf of IAA (Nichols 1958). The problem with cacao is the difficulty of extracting the active labile compounds present in low concentration and, therefore, critical bioassay after repeated chromatography is not as easy as with other plant species which have been examined.

Examination of terminal buds from fan branches in the resting stage

The hypothesis that "flushing," the rapid growth of fan branches after a period of dormancy, might be triggered by an accumulation of auxin was well worth examination. It had been found by field investigations that cacao trees do not necessarily "flush" at the same time and that certain trees within a group of identical clonal material might maintain a flushing cycle quite independent of their neighbours. The time lag might be only a matter of days between them, but this would be critical if one were looking for an accumulation of an unknown but growth-active substance which was present in detectable amounts for a limited period. That is, as soon as active growth started a depletion of the active substances might occur. However, it was shown that if individual fan branches were tagged at a relatively easily recognisable *physiological* stage in development, these branches could be confidently expected to flush together at the next flushing period. A convenient stage to recognise is when the flushes are losing their red pigmentation and the leaves are assuming a reddish-green hue. At this time the leaves are still relatively flaccid and unhardened. The bud, with its scale leaves and a $\frac{1}{2}$ in. of the stem, terminal to the tagged flush is used for subsequent extraction. Twelve hundred such branches were tagged in late October and sampled at various dates. For each extraction, 150 buds were selected having an approximate fresh weight between 19 and 24 gms. They were collected, frozen in dry ice and freeze dried. Samples were taken at 0, 11, 18, 31, 44 and 62 days from tagging; a week later the remaining buds were showing signs of bud-break.

With one exception, all the bud extracts were devoid of substances active in the curvature test; the exception was the sample taken at 18 days from tagging which showed a barely significant curvature, equivalent to <0.05 IAA equivalents per litre.

From earlier work it had been shown that the acidic inhibitor was present in bud extracts and if this were present in excess it might suppress the activity of either auxin I or II. Chromatographic analysis of the bud extracts did not reveal any inhibition due to the acidic inhibitor in the curvature test from which it may be inferred that the inhibitor does not pass from the agar into the coleoptiles. In order to confirm this observation bud extracts were prepared and synthetic IAA was added to make a final concentration in agar of 0.12 mgms./l. Similarly agar containing only synthetic IAA to a concentration of 0.12 mgms./l. was prepared. Both agar samples were tested and gave respectively mean curvatures of 13.5° and 15.4°, an insignificant difference. A significant depression of the curvature would be expected if the inhibitor were readily translocated from the agar into the coleoptile. Clearly, the lack of curvature is then due to the absence of auxin rather than a surfeit of inhibitor. This is not to exclude the strong possibility that the presence of an inhibitor is the determining factor in the dormancy of the buds; it is merely not detected in the oat curvature test.

Discussion

From the results presented, it may be seen that wilting of pods is related to endogenous auxin production. However, it is difficult at present to understand how this control is exerted and how the wilting behaviour is related to nutritional and water stresses. Clearly no single factor is paramount and only by providing optimal conditions for some of the factors particularly water and nutrients may the overriding effects of another be expressed. Under field conditions, it is extremely difficult to obtain optimal conditions of any factor and, therefore, field experimentation with synthetic growth regulators is at once difficult to interpret. Assuming that the hypothesis of hormonal control of wilt is correct then control of wilt by growth regulators is conceivable. Under *strictly specified* conditions Akinbode (op. cit.) was able to achieve control with 2 : 4, Dichlorphenoxy acetic acid, but failed to repeat the experiment at a different time of the year. He also showed that the waves of wilting were different in respect to the ages of the cherelles at different times of the year. It is not then surprising that his control of wilt gave different results at differing seasons.

The immediate problem remains that it is still not known how the endogenous auxins control wilt in the absence of an abscission layer; if this factor can be determined a major step forward will have been obtained.

The work on bud dormancy is interesting as it indirectly bears out current views on tissue dormancy. It is more likely that a removal of an inhibitor rather than an accumulation or depletion of growth accelerator is responsible for dormancy in buds. Having established that dormant buds of fan branches of cacao do not contain readily detectable auxins it will be relatively easy to examine the buds with the straight growth test without having recourse to elaborate purification techniques.

Summary

(1) A correlation between cacao auxins I and II and wilting of cherelles has been established and further work done on the nature of auxin I has confirmed that it is probably not IAA.

(2) Dormant buds of fan branches have shown virtually no residual auxins between flushes.

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Examination of culture filtrates of *Marasmius perniciosus* Stahel for gibberellin-like substances

by

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Cacao plants infected with *Marasmius perniciosus* produce excessive and abnormal branching which has given rise to the descriptive name Witches' broom disease. It has been suggested by Brian (1957) that such symptoms may be caused by the production of growth-stimulatory or auxin-like substances. During investigations on the effect of gibberellic acid (GA) on cacao seedlings (Nichols, 1959) certain morphological similarities were observed between the effect of GA on seedlings and the appearance of cacao shoots infected by *Marasmius*. It was, therefore, of some considerable value to find out whether the "brooming" of cacao shoots was induced by substances of the gibberellin-type, i.e. whether *M. perniciosus* produced these substances *in vivo*.

The technique employed was to grow freshly isolated mycelia of *M. perniciosus* in culture solution and to apply the concentrated filtrates to cacao and rice seedlings. Cacao seedlings were an obvious choice as test plants, but rice seedlings were also used because it was known and confirmed by experiment that they respond very markedly to GA solutions. It was conceivable that gibberellin (or GA) might be but one of a complex of substances which promote "brooming" and, therefore, it was necessary to use a test plant such as rice which was shown to be more responsive to GA in low concentrations than cacao seedlings. Plants treated with the uninoculated medium were used as controls, and plants treated with culture filtrates of *Fusarium moniliforme* grown in the same medium were used as an index of the production and behaviour of GA.

In order to prepare culture filtrates, the organisms were grown on the following medium which contained per litre : hydrolysed molasses, 20.6 gms. total sugar ; $(NH_4)_2SO_4$, 3 gms. ; KH_2PO_4, 3 gms. ; "marmite," 5 gms. The medium was adjusted to pH 6.3 and dispensed in 100 ml. amounts in indented 250 ml. conical flasks. The cultures made good growth when grown on a rotary shaker at tropical room temperature (26-29°C.) for 20 days by which time sugar utilisation was complete (97%). The cultures (20 flasks) of each organism were combined and filtered through sintered glass after which the sugar concentration in the clear filtrates was restored to that in the uninoculated medium by the addition of equal amounts of glucose and fructose. The pH was also adjusted to its initial value. The culture filtrates and control medium were concentrated eightfold by evaporation under reduced pressure at 40°C.

The effect of the concentrated filtrates on growth was examined by the application of 0.1 ml. to the terminal buds of cacao seedlings grown in seed boxes about 4 weeks from germination. Each solution was tested on 12 plants and the plants were treated twice weekly at 3 and 4 day intervals for a total period of 4 weeks. Concentrated and unconcentrated filtrates were tested in this manner. The *Fusarium* filtrates gave a marked elongation of the stem by typical internode elongation compared with the control plants, but no effect either stimulatory or inhibitory was observed with the *Marasmius* filtrates. Similar results were obtained when the filtrates were applied to rice seedlings.

Despite the negative results with cultures grown *in vitro*, it remained possible that the substances responsible for the growth abnormalities of cacao were produced by the *Marasmius in vivo* in the host plant. This possibility was examined by treating seedlings with an extract of infected cacao tissue. This extract was prepared by macerating freshly collected Witches' broom (250 gms.) in a Waring blender with a little water and expressing the mucilaginous pulp through several layers of cotton gauze. The filtrate was concentrated approximately ten-fold by evaporation to a thick gel in a freeze-drying apparatus. Cacao seedlings were treated by application of the extract to the stem just below the terminal bud. Some of the seedlings were pricked after the initial application to ensure entry of the extract into the plants. Treatment was continued twice weekly for 4 weeks. The extract had no effect whatsoever on the growth rate relative to untreated control plants. These experiments suggest that *M. perniciosus* does not produce a detectable growth-stimulatory substance similar to gibberellic acid under the conditions of the culture and test whereas *F. moniliforme* does.

The strain of *F. moniliforme* (NRRL 2284) was kindly supplied by Dr. C. W. Hesseltine and the GA by Dr. P. W. Brian.

* Dr. W. F. Dudman, Colonial Microbiological Research Institute, Port-of-Spain.

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The Efficiency of a Test of the Resistance of Cacao Seedlings to *Marasmius perniciosus* Stahel

by

B. G. D. Bartley

In order to determine the resistance reactions of different cacao genotypes to Witches' broom disease, caused by *Marasmius perniciosus* Stahel, without having to rely on long-term field tests, it was necessary to develop an inoculation test which could be employed in the greenhouse and which would provide a method of comparison of the types of resistance possessed by different individuals. An inoculation technique which involves dipping four-day-old seedlings in aqueous suspension of basidiospores was described by Holliday (1955) and later by Baker and Holliday (1957). During the succeeding years a larger range of genotypes has been tested and observations made on the reactions of the plants to artificial infection in order to improve the efficiency of classification of degrees of resistance or susceptibility.

The technique described by Holliday (1955) has been used throughout, with few modifications. The major change is that the inoculations are done in the morning instead of at night as the former is a more convenient time and more spores are available. Every effort is made to complete the inoculations by 8.30 a.m., and the results are similar to those obtained when the inoculations are done at night.

In order to compare the symptoms produced by individual plants at various times a scoring system has been introduced. Scores are given from 0 to 4 for each of the zones where hypertrophy occurs, the scores given being dependent on whether hypertrophy is absent or present and the degree of hypertrophy. The heights of the plants are measured during the assessment periods in order to obtain an estimate of the comparative growth rates of the plants being tested.

Reactions to Inoculations

A proper understanding of the problems involved in the classification of inoculated plants may be obtained by a study of the reactions of the plants to the fungus and of the relationships among these reactions and the environmental and other factors which contribute to the overall expression of symptoms. The key to the efficient use of the inoculation test is in the development of a sensitive method of classifying the plants that have been subjected to artificial inoculation, since an apparently continuous scale of symptom expression occurs.

One effect of infection is the retardation of growth in some of the more susceptible seedlings, the development of the second flush being affected. At two months of age the average height of the inoculated plants is slightly less than the average height of the non-inoculated plants of the same cross. At three months the difference in height is usually very well marked. Tables I and II present the average heights of inoculated and non-inoculated plants of several crosses at two and three months of age and the increase in height that occurred during this period. The increase in height of the inoculated plants of the cross SCA 6×IMC 67, which apparently contains a large proportion of resistant plants, is proportionately more than the height increases among other crosses such as ICS 6×IMC 67, where there is a high percentage of susceptible plants. In plants that survive the inoculation test, growth is generally continued after a few weeks and they develop normally after planting in the field.

Another reaction of some inoculated plants to the fungus is the production of necrosis and the death of the plant. The lethal effect is very pronounced in susceptible populations. In most of the crosses tested the largest percentage of deaths has occurred between two and three months of age, as shown in the mortality figures in Tables I and II. A few deaths occur after three months. Where the proportion of such plants is high, this is probably due to a delayed incubation period.

In some susceptible material, such as selfed plants of M 8, as much as 50% of the infected plants die before reaching an age of two months and all plants die before three months. In other plants which appear to be equally susceptible no deaths occur before two months, but mortality is complete at three months. The latter plants grow more vigorously and it would appear that the death rate is dependent on the inherent vigour of the plants being tested ; so that the death rate is no indication of the relative susceptibility of plants which carry different genes for vigour. A 100% mortality rate has been recorded in several cases such as M 8 selfed, ICS 1×ICS 8, ICS 1×M 8 and ICS 8×M 8. In crosses involving resistant types such as SCA 6 no deaths may occur, but a small percentage of deaths has been recorded in some crosses involving SCA 6, especially those with ICS 8, ICS 36, ICS 43, ICS 95 and M 8. A relationship appears to exist between the mortality rate and the degree of susceptibility of the parents.

The relationships between the other symptoms which are developed and the lethal effect are not always well defined. Death, in the most susceptible types, usually follows the development of severe hypertrophy, but severely hypertrophied plants do not die in every case, neither is the growth of susceptible seedlings always checked when the hypertrophy is severe. While the majority of plants which eventually die do not develop further after two months of age, in certain cases death

TABLE I

Symptom classes, mortality rates and relative growth rates of inoculated seedlings of the cross ICS 60×SCA 12.

Replication	Per cent survivors in classes			Per cent dead at months			Total in class	Average heights of plants in cms. Inoculated months			Controls months		
	I	II	III	2	3	4	III*	2	3	Increase	2	3	Increase
1	14	31	30	0	19	24	54	23.2	29.7	6.5	25.5	42.5	17.0
2	18	56	15	0	12	12	27	21.7	24.1	2.4	22.7	26.1	3.4
3	3	64	13	0	15	20	33	21.2	22.7	1.5	23.0	31.1	8.1
4	9	59	12	0	20	20	32	20.0	21.0	1.0	21.4	23.7	2.3

* Total in class III includes percentage of survivors in class III plus percentage dead at four months.

TABLE II

Percentages in symptom classes and relative growth rates of inoculated and control seedlings of several crosses involving IMC 57 and IMC 67.

Cross	Per cent survivors in classes			Per cent dead at months			Total in class	Average heights of plants in cms. Inoculated months			Controls months		
	I	II	III	2	3	4	III	2	3	Increase	2	3	Increase
ICS 1×IMC 57	0	35	47	2	17	18	65	21.4	23.6	2.2	22.0	35.5	13.5
	0	1	62	0	32	37	99	24.7	27.8	3.1	25.0	37.1	12.1
ICS 1×IMC 67	0	33	22	10	43	45	67	23.2	24.0	0.8	24.6	31.3	6.7
	3	38	53	0	5	6	59	24.7	25.7	1.0	25.6	34.6	9.0
ICS 6×IMC 67	0	32	50	1	16	18	68	23.3	24.2	0.9	24.9	27.9	3.0
	5	33	52	0	3	10	62	23.5	26.6	3.1	26.2	35.1	8.9
ICS 89×IMC 67	0	16	30	1	51	54	84	17.2	18.7	1.5	20.0	31.7	11.7
ICS 60×IMC 67	6	39	11	8	39	44	55	24.4	25.6	1.2	24.0	31.2	7.2
	0	8	22	8	70	70	92	22.5	23.1	0.6	22.5	25.1	2.6
	10	34	34	1	20	22	56	23.2	24.3	1.1	24.1	32.4	8.3
SCA 6×IMC 67	18	56	26	0	0	0	26	19.2	26.1	6.9	20.8	29.6	8.8
	4	72	24	0	0	0	24	18.0	20.5	2.5	18.9	23.6	4.7

may occur after new growth has been produced. In a large number of instances death has occurred in plants showing no symptoms or only very slight symptoms. On the other hand, there have been some striking examples of seedlings, producing very well developed symptoms, but growing as well as the non-inoculated plants. In less vigorous susceptible plants death may occur so rapidly that the other symptoms are not observed.

In addition to the variation in symptom development due to the inherent vigour of the plants being tested, environmental conditions influence the degree of hypertrophy and the mortality rate by their effects on plant development. Holliday (1955) observed that variations in growth rate of the inoculated plants contributed to the inconsistencies in symptom development and difficulties in symptom assessment.

The heights and average growth rates of the control plants serve to measure the deviations in environmental conditions and of the relative vigour of various types of plant material. The average heights at two and three months of the controls of several crosses, which are given in Tables I and II, demonstrate that fairly wide differences in environmental conditions occur when the growth rates of the replications are compared. The results of inoculations on plants of the cross ICS 60× SCA 12 (Table II) show a wide disagreement in the symptom classes and the relative mortality rates. In this case the replication with the best growth rate had the highest proportion of Class III plants and the highest mortality rate. On the other hand, in three of the crosses involving IMC 67 the replications having the lowest average growth rates also had the highest mortality rates. In the cross, ICS 60×IMC 67, where the results from three replications are given, there is a consistent relationship between the growth rate and the mortality rate, there being more deaths when growth conditions are unfavourable.

Efficiency of classification

Two aspects should be taken into consideration when the reliability of the inoculation test is discussed. The test should be able to distinguish the same reactions of plants under all conditions. In this respect different replications of the same genetic material should give similar results.

The second point is the relationship between the reactions expressed under artificial infection and those observed under natural infection.

From the data given in Tables I and II it appears that the results from different replications of the same crosses neither coincide with regard to the various degrees of hypertrophy developed nor to the mortality rate. This results in different proportions of plants being placed in the three classes. While one replication of the cross, ICS 1×IMC 57, suggests that some of the progeny are resistant the other shows that all, or at least 99%, are susceptible. Since large numbers of seedlings were inoculated in each case, it is improbable that the discrepancies observed were due to differences in the genetic constitution of the plants in each replication. More concordant results, in some crosses, have been obtained from different replications, although some discrepancies still exist. In the cross, ICS 1×IMC 67, the percentage of Class III plants was the same in both replications although the percentage mortality was very different. It is obvious from these results that inferences as to the relative degree of susceptibility of the parents of the crosses, or of the crosses themselves, cannot be made with certainty in the absence of replication.

From the few results that are available from inoculated plants later subjected to further infections under natural conditions, it is evident that the classification of plants that has been carried out previously does not coincide with the results of natural infection. Of the trees of the cross, ICS 60×SCA 12, which have been grown at River Estate since 1951, 40% have produced brooms so far (Bartley 1958). From the figures obtained in the most heavily infected plots it would appear that the actual proportion of susceptible plants will be over 50%. Only one of the replications of this cross artificially tested (Table II) shows a similar proportion of Class III plants, the proportions in the other replications being below 40%. In another cross involving SCA 12 (that with ICS 1) the proportion of artificially inoculated seedlings in Class III as determined by Holliday (1955) is 21%; but about 35% of the seedlings of this cross which have been exposed to natural infection have produced brooms. Table III compares the classification by artificial infection to infection so far recorded on the same trees of this cross under natural conditions. Class II contains a high proportion of susceptible trees. One plant on which no apparent symptoms were produced in the greenhouse has been infected in the field. In several other crosses seedlings showing no symptoms when artificially inoculated have become infected in the field. The probable reason for this occurrence is the failure of artificial inoculation to infect the seedlings. The general tendency of the system of classification used so far is to put too few plants in the susceptible category when segregating populations are examined for resistance.

One group of artificially inoculated plants which showed a wide divergence from the field reaction were those from the cross ICS 6×ICS 8. From the results obtained from other crosses involving these two clones it was expected that all progeny of this cross would show a highly susceptible reaction and this is developed in the field. Two replications of plants of this cross were inoculated and a mortality rate of only 20% was obtained, and about half of the plants produced only slight hypertrophy although there was an infection rate of 100%. Among

TABLE III

Comparison of artificial inoculation classes and field infection among the progeny of ICS 1×SCA 12

Artificial infection classes	Number of plants	Number infected naturally
I	11	1
II	9	6
III	8	8

the plants of this cross there was a marked suppression of growth, the average increase in height during the third month being only about 15% of that of the control plants. Similar results were obtained from the two replications.

Improving efficiency

It is evident from the information given above that the classification of individuals in segregating populations must be made more efficient in order to reduce inconsistencies and to provide a more precise comparison with results obtained under natural infection. The most outstanding causes of the inconsistencies observed are failure to achieve infection, insufficient infection and variations in the normal reaction to infection due to changes in the environment to which the plants are subject during the period of the test. Failure of infection is indicated by the presence of unexpectedly large proportions of symptomless plants. The only apparent remedy for insufficient infection is to provide an adequate inoculum potential by obtaining a large and continuous supply of spores so that a concentration of viable spores above that necessary to produce certain infection can be used for the inoculations. It is important also that the inoculations be carried out within a standard interval from the initiation of spore deposition. These conditions may not provide the whole solution since we are virtually ignorant of the existence of strains of *Marasmius perniciosus* of varying pathogenicity, or of the conditions required for maximum infection.

Most of the reasons for using the test for resistance involve comparisons of reactions, and in order to permit accuracy in these comparisons it is desirable to reduce the effects of the environmental changes and to maintain uniform conditions for the growth of the plants under test. Optimum conditions for growth are also desirable to obtain the maximum expression of the symptoms. Only under these conditions will it be possible to classify the seedlings accurately at an early age and make reliable classifications. When the test is used for screening the susceptible plants uncertainties in classifying the plants will result in a larger proportion of plants being kept for field testing, and the expense of the breeding programme will be correspondingly increased.

Owing to variations in environmental conditions, differences in symptom pattern occur during the life of the plants and it is necessary to examine the symptoms at various stages. The introduction of the scoring system has been very useful in following the development of the symptoms. Scores are given when the seedlings are two and three months of age, these periods corresponding roughly to the hardening of the first and second flushes, respectively. During the preliminary assessment scores are given on the basis of the presence of or severity of the hypertrophy and the heights of the individual plants are

taken into consideration in order to provide some correction for differences in the inherent vigour of the individuals comprising a population. At three months of age the differences in development of the plants are sufficiently well marked to permit a further assessment. At this stage it is possible to determine whether growth conditions have been satisfactory by a consideration of the average increase in the heights of the control plants and this is borne in mind when the inoculated plants are classified. When growth conditions are suitable little difficulty is experienced in sorting the plants according to their symptoms and the extent to which growth has been checked. Small increases in the heights of the controls indicate unfavourable conditions for growth and it is difficult to distinguish between the appearance of the controls and the inoculated plants, and accurate classification of the latter is not always possible. In some groups of artificially inoculated plants the development of hypertrophy occurs slowly due to delayed incubation of the fungus. In one case as many as 25% of the seedlings developed hypertrophy of the hypocotyl only after two months. The development of symptoms, however, is usually complete at about three months from the inoculation date and it is felt that the final classification can be made by an experienced person at this time. This classification should be accurate provided that conditions for symptom development have been suitable. This will reduce the time that the plants are under examination as compared with the four to six months previously suggested and will result in a greater usefulness of the test and an economy in its operation.

Owing to the variability in environmental conditions that has been experienced so far it has been found necessary to test at least three replications of one cross in order to obtain a reliable analysis of plant reaction. This requirement results in much extra work and consequent delay in testing other types. From comparisons among the replications of a cross it appears that the most reliable interpretation of the reaction to the disease is obtained from the replication that gives the largest percentage of Class III plants and the best pattern of symptom development.

All the inoculations done so far indicate that the resistance test is generally capable of distinguishing between the most susceptible and most resistant types, but is not sufficiently precise in cases where the reaction is intermediate and variable. Under most conditions a sufficient degree of sensitivity can be realised to make it possible to screen some of the most susceptible segregants. The test has some advantage over methods employing natural infection where there is a similar effect of the environment and vigour of the individuals on the expression of the symptoms and where time and space are limited. Where it is intended to compare the reaction of individual plants in a cross, as for studies of the mode of inheritance of the factors determining resistance, the uncertainty of obtaining an accurate and definite pattern of symptom expression gives the artificial methods little advantage over the field tests with respect to reliability. In spite of the difficulties encountered in classifying the artificially inoculated plants, experience has shown that this method is as suitable a method of obtaining the desired results as field tests alone, although exposure of artificially inoculated plants to natural infection must be used as an accessory method of analysis of resistance.

Summary

In segregating populations subjected to artificial infection by *Marasmius perniciosus* a range of symptom expression occurs. Different replications of these populations often give very different results. Variations in environmental conditions which affect the growth of the test plants influence symptom expression and accurate classification of phenotypes is obtained only when growth conditions are uniform. The use of growth measurements and a system of scoring for hypertrophy of individual infected plants aids assessment when environmental conditions are not controlled.

Comparison with natural infection results indicates a tendency for the previous system of assessment to place too few plants in the susceptible class.

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A Note on the Infection of *Herrania* Species by *Marasmius perniciosus* Stahel

by

B. G. D. Bartley and E. F. Iton

THE genera *Herrania* and *Theobroma* possess certain features in common, but although some authorities dispense with the generic ranking of *Herrania* and include its members as species of *Theobroma*, the former is easily distinguishable from the latter on the basis of habit. *Herrania* is, therefore, treated as a separate genus by Schultes (1958) who describes the distinguishing characters of the two genera. On account of its close relationship with *Theobroma*, *Herrania* is receiving some attention as a subject for genetic and other studies, including disease resistance.

FIGURE 1
Two necrotic " cushions " brooms from *Herrania purpurea*.

FIGURE 2
Necrotic lateral vegetative broom on *Herrania nitida*.

Herrania is indigenous to Central America and northern South America and its distribution coincides with that of *Theobroma*. Some species of *Herrania* occur naturally in areas of South America where Witches' broom disease is found to infect *Theobroma cacao* and other *Theobroma* species. However, the only report of infection on *Herrania* was made by Pound (1938) who observed cushion infection on two trees of an unidentified species growing in a block of cacao trees on the Rio Orteguaza. The Anglo-Colombian Cacao Collecting Expedition, a report on which is given by Baker *et al.* (1954), found no evidence of Witches' broom infection on *Herrania* in the Amazonian region of Colombia. Baker and Holliday (1957) do not mention *Herrania* as a host for *Marasmius perniciosus* Stahel.

A collection of *Herrania* species has been established at River Estate. Most of the material for this collection was

introduced as a result of the Anglo-Colombian Cacao Collecting Expedition. At the present time the collection consists of ten species which are identified and named according to the descriptions given in the synopsis of the genus by Schultes (1958).

Witches' broom infection was first observed on *Herrania* trees in this collection in 1956. So far, vegetative and floral infection have been observed on three species, *Herrania albiflora*, *H. purpurea* and *H. nitida*. It is of interest to note that the first two species occur naturally in areas where *Marasmius perniciosus* has not been found and the last named species is the most common species in the Amazonian region where the disease is prevalent. Diseased fruit of *Herrania albiflora* was found to contain mycelium of *M. perniciosus*. In addition, floral infection has been observed on the interspecific hybrids, *H. albiflora* $\times$*H. nitida*, and *H. albiflora*$\times$*H. balaensis*.

Infection in the axils of leaf scars (i.e. on the older parts of the stem) gives rise to cushion brooms. Fig. 1 shows two typical examples of such brooms. They consist of many green closely-bunched hypertrophied vegetative shoots which bear only small leaves and sometimes produce flowers. Terminal buds and axillary buds on the younger parts of the stem are also infected and give rise to green hypertrophied shoots. Where terminal buds are infected the internodes generally are shorter than normal and the pulvini of the leaves are much enlarged. Necrosis takes place in all cases of infection and the characteristic pink sporophores are produced on the necrotic tissue. An example of a necrotic " lateral " vegetative broom is shown in Fig. 2. In the case of small plants with single stems terminal bud infection may result in the death of the plant.

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Studies on a Wilt Disease of Cacao at River Estate

by

E. F. Iton

Introduction

A wilt disease caused by *Ceratostomella fimbriata* (Ell. & Hals.) Elliot* is known in cacao in Costa Rica (Alston 1925), in Colombia (Garces 1944), in Venezuela (Malaguti 1952) and in Ecuador (Desrosiers 1958).

In March, 1958, several planters reported rapid wilting and death of cacao trees on estates in the Northern and Central Ranges of Trinidad. The symptoms resembled those of *Ceratostomella* wilt in Central and South America and the Colonial Microbiological Research Institute, working in collaboration with the Department of Agriculture of Trinidad and Tobago, established that *C. fimbriata*, in association with shot-hole borers of *Xyleborus spp.*, was the cause of the disease.

The fungus *C. fimbriata* is virtually world-wide in its distribution and has been reported from several countries as the cause of diseases in rubber, coffee, coconuts and sweet potatoes (Chevaugeon 1957). It has been reported in Trinidad on *Crotalaria juncea* (Briant 1932) and as a minor pod rot of cacao (Baker 1936), but it has never been known to cause any disease of the vegetative organs of cacao locally. However, this new wilt disease broke out simultaneously in widely separated cacao estates in Trinidad, and a field survey conducted by the Department of Agriculture of Trinidad and Tobago (1959) shows that the disease now occurs in many parts of the island (Fig. 1).

Shortly after being first reported the disease appeared at River Estate in the experimental fields of the Regional Research Centre and close field studies were begun. This paper presents some of the findings of these studies. It must be emphasised that many aspects of the problem of this disease are still unsolved and conclusions reached here might need modification or even alteration in the light of new knowledge.

Symptoms

The external symptoms of the disease are wilting of the whole tree or part of it followed by rapid death of the affected parts. In the earliest stage of wilting the mature leaves change from the usual semi-erect or horizontal attitude to a pendulous one similar to that of a young flush. These leaves are at first a normal green, but within a few days they begin yellowing or browning irregularly over the surface. Finally, often in a matter of days, the leaves become dry, brown and more or less longitudinally rolled (Fig. 2). In this condition they remain on the dead branch or tree for at least several weeks. The pods also shrivel, but less rapidly than the leaves.

These symptoms are invariably associated with borings of the trunk or branch of the tree. The holes, which are made and inhabited by *Xyleborus spp.*, are about 1 mm. in diameter and penetrate the bark and wood more or less at right angles to the axis of the stem (Fig.3a and b). Small coiled plugs of frass protrude from them. Roots of diseased trees removed from the soil contain the shot-hole borers and also have borings with frass plugs (Fig. 4) which indicate subterranean activity by the borer.

The extent of the wilting appears to be related to the siting of the borings. When the shot-holes are on the root or the trunk the whole tree dies, while borings restricted to the branches are associated with death of the infested branches only. Apparently symptom expression occurs only distal to the level of infection which coincides approximately with the lowest level of borer infestation. Whether infection or infestation comes first is not known. Observations indicate that infection can spread downwards in the wood from the initial points, but the rate at which this generally occurs and the final extent of such downward spread are unknown. Some trees from which large infected branches have been cut have, after a period of several months, shown no further symptoms, and sometimes chupons grow out from below the level of the borings on trees which are quite dead above the jorquette.

The incubation period of the disease is unknown. Some trees have their trunks badly bored for several months before showing any symptoms of the disease. Further observations are necessary to determine whether the incubation period is usually protracted, or whether boring does in fact have any direct relation with the disease.

The internal picture in the diseased tissues is also very striking. Slices of bark taken tangentially in the region of the borings reveal a brown or claret discoloration which is accompanied by a smell strongly resembling that of sardines. Sections through the wood show usually a blue-grey, sometimes a dark brown and very occasionally a yellow discoloration. The proportion of discoloured wood to healthy wood progressively decreases with increasing distance from the borings, passing from a continuous expanse to discrete patches lenticular in transverse section (Fig. 5a, b and c) and finally fading into normal wood.

Laboratory studies

Isolations from both the discoloured wood and the healthy-looking wood near this have consistently produced

* The correct name is *Ceratocystis fimbriata* (Ell. and Hals), but this fungus is better known by the name used in this paper.

FIGURE 1
Map of Trinidad showing cacao areas and the distribution of the disease.

FIGURE 2
Diseased cacao tree showing dry leaves.

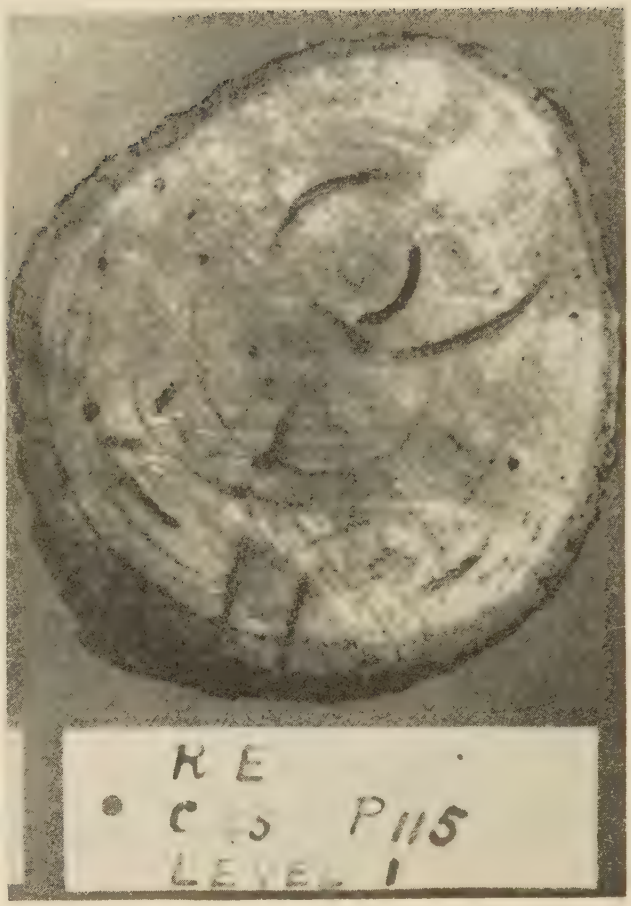

FIGURE 3a
(*a*) Transverse and (*b*) longitudinal sections of diseased trunk showing *Xyleborus* borings.

FIGURE 3b

FIGURE 4
Diseased cacao root showing frass coils extruded by the insects.

Fusarium spp. and *C. fimbriata*. Artificial inoculations made with pure cultures of the *Fusarium* spp. have, so far, produced no symptoms in the host plants. Similar inoculations with pure cultures of *C. fimbriata* have, however, produced the symptoms and death described in 11.8% of the 34 cuttings inoculated. The corresponding number of control plants has remained healthy so far.

The discoloured wood, the walls of the borer-galleries, and the frass extruded by the borers show, on microscopic examination, numerous intracellular chlamydospores of *C. fimbriata*. Chlamydospores are numerous in the artificially inoculated plants which die and also in those which show no symptoms. Endoconidia and perithecia are easily produced on wood from diseased trees and in agar culture, but chlamydospores are formed only in small numbers in agar culture.

Epidemiology

Incidence

The availability of precise information on the genetical constitution, age and history of the individual trees in the experiments at River Estate makes this area well suited for field studies and a detailed survey has been made.

River Estate has some 240 acres under a total of some 87,000 cacao trees of various ages. Although, on the whole, younger trees appear to be less frequently attacked, trees of all ages have been killed by the disease. The incidence of disease, i.e., the number of diseased trees in relation to the total number of trees as at 31st January, 1959, was 0.7%.

Transmission

The first diseased tree at River Estate was recorded in March, 1958, in field 22B (Fig. 6). In May the disease appeared in field 19. In June it reached field 9C. Shortly afterwards outbreaks occurred almost simultaneously in several different fields in the estate.

Usually in epidemics of wind dispersed diseases the distance over which appreciable quantities of inoculum from a single source of infection are transmitted are a matter of yards. The maximum range over which the minimal quantity of inoculum can be transmitted from the same source might, however, be many miles. The result is that although the immediate economic loss is small at great distances from the original source, yet the possibility of establishing isolated new foci of infection does exist. Subsequently the gaps in the continuity of diseased tracts are filled up by coalescence of new areas of infection generated from these new foci.

This was the basic pattern at River Estate. By December the disease was widespread in the estate. The original foci had expanded, the larger ones more rapidly than the smaller, with the result that the earlier infected fields generally had a higher disease incidence as Fig. 6 shows.

Fig. 6 also shows a definite north-east to south-west trend of disease across the estate. When this spatial distribution is coupled with the sequence of disease occurrence in fields 22B, 19 and 9C described above, it strongly indicates that inoculum has been transmitted from field 22B to field 19 and then from 19 to 9C by the prevailing north-east trade winds. The distribution of the disease within the separate fields is also strongly north-east to south-west although this trend is now obscured in field 22B. The obscuring of this pattern in field 22B is due to the fact that the disease has been longest established in that field and, therefore, the coalescence of infected areas has had time to occur, especially as this field has a high percentage of the clone ICS 45 which, this study later reveals, is among the most susceptible cacao material at River Estate.

Although it is possible that insects contaminated with the infective organism could travel considerable distances when aided by the wind, the extreme susceptibility to desiccation of the *Xyleborus* borers associated with this disease makes it unlikely that they play an effective part in long-distance transmission of the disease. In the laboratory, on several occasions, all the beetles in batches of twenty to twenty-five survived a maximum period of one day when kept in a dry petri dish or jar, but a large percentage lived actively for twelve to fourteen days when placed in a jar with wet filter paper or sand. It is, therefore, not unlikely that these insects can be effective vectors in short-range disease transmission.

The field observations do not prove conclusively that wind is the only factor involved in the transmission of the disease at River Estate. Also the fact that *C. fimbriata* usually behaves as a wound parasite raises some difficulty in explaining this spread of the disease on the basis of wind transmission alone. Possibly wind-blown insects were responsible for the rapid establishment of foci of infection in the different fields within the estate.

FIGURE 5a

FIGURE 5b

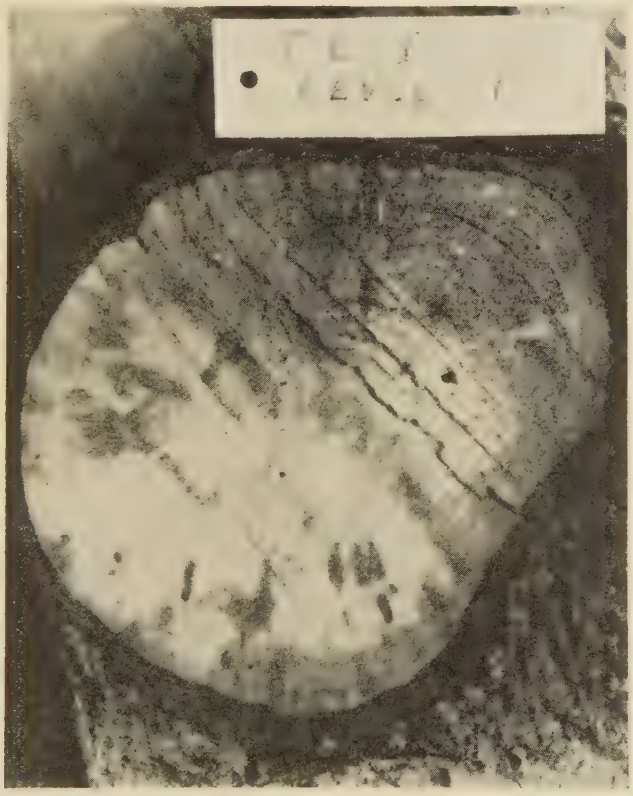

FIGURE 5c

Transverse sections of diseased cacao trunks showing decreasing continuity of discoloured wood with increasing distance from insect borings.

FIGURE 6
Map of River Estate showing incidence of *Ceratostomella* wilt.

The basic north-east to south-west trend cannot be explained by transmission of the disease from tree to tree by contaminated agricultural implements. It might be suggested that inoculum is universal and that this trend coincides with the distribution of the highly susceptible cacao at River Estate. Such a coincidence of highly susceptible host plants with the trend of the disease does not exist. On the contrary, several plots of very susceptible material have escaped infection in fields where the overall disease incidence is high. The advance of the disease from north-east to south-west also precludes this possibility. Lastly, no proneness to disease induced by soil types can be put forward to fit this basic pattern because the trend cuts across different soil types.

The evidence, therefore, indicates that wind is involved in the transmission of the disease. It is uncertain as to whether field 22B was the focus of infection for the entire estate. The possibility of infection from a source or sources external to the estate cannot be ruled out.

To help determine further the part played by wind in the spread of this disease, the air spora was sampled at heights ranging from one foot to six feet above soil level and at three feet to six feet from the trunk of one diseased tree in each of three places at River Estate. The method used was the exposure of vaselined slides surrounding each tree and fixed so that the greased surfaces were directed towards the tree. The final counts of spores so collected have not been completed, but spores of several fungi which are usually to be found in the tissues of the diseased cacao plants have been trapped. Among these are *Fusarium spp.* and chlamydospores of the same morphology and order of size as those to be found in the tissues lining the borer galleries in naturally infected trees, in the frass extruded from these galleries, and in plants artificially inoculated with pure cultures of *C. fimbriata*. These chlamydospores are not found free on the slides, but in large numbers embedded intracellularly in microscopic bits of wood similar to the frass produced by *Xyleborus* spp. on cacao trees.

This observation might possibly lead to an explanation of the association of high borer incidence with high disease incidence, the former perhaps being indirectly necessary for the production of a large inoculum potential for wind dispersal. Nor is it contradictory to the phenomenon noted about the relation between borer siting and extent of wilting in a given tree, if one assumes that infection occurs as a result of wind transmission of the pathogen and subsequently the borers colonise the infected parts before any visible symptoms appear in the host. So far it is not known with certainty whether infection precedes infestation or vice versa in Trinidad. In Colombia Naundorf *et al* (1956) have established that the *Xyleborus* borers are vectors of the disease, whereas Malaguti (1958) has found that although they occur in large numbers in diseased cacao in Venezuela they are not the effective vectors of this disease.

At River Estate, subsequent to the establishment of the initial foci of infection in the different fields, the short-range spread of disease was in all directions from each focus. It is, therefore, probable that insect vectors were effective at this stage of the epidemic. Attempts to isolate *C. fimbriata* from the insects have failed on several occasions, but this cannot be interpreted as evidence against the insects as vectors.

Whether as direct or indirect agents in the spread of the disease, these *Xyleborus* borers of cacao appear to be important, and, therefore, a search has been made in and around River Estate for alternative hosts and breeding grounds for the beetles. So far they have been found in fallen Immortelle (*Erythrina* sp.) trunks and in living and dead trunks of a *Gliricidia* sp.

Resistance

In the relatively short time since the disease broke out at River Estate all the cacao has not been uniformly exposed to infection and, therefore, final conclusions cannot be drawn on the relative susceptibility of different clones or seedlings. Nevertheless, some observations on this aspect of the problem are of interest. Table I summarises the position with regard to certain clones in fields 7, 9C, 6A, 19 and 22B (Fig. 6) which have populations of 4,882, 4,140, 3,476, 8,423 and 2,208 trees, respectively.

The figures suggest that ICS 38 and 45 are very susceptible and ICS 39 and 60 are less susceptible, whereas ICS 1, 6, 8, 40, 95, 98 and SCA 6 and 12 are resistant. Of the susceptible clones ICS 45 is remarkable in that it is largely responsible for the very high incidence of disease in field 22B.

Of the resistant clones ICS 95 is outstanding considering that its high population of 639 trees shows an incidence of only 0.9% in a field where the overall incidence is 2.6%, and similarly its population of 241 trees shows an incidence of only 0.4% in a field with an incidence of 0.9%. ICS 1 is also worth mentioning in view of the fact that its considerable population of trees shows no disease in field 22B which has a relatively long established epidemic and high disease incidence of 3.2%. Further, the very low disease incidence of 0.3% in field 20A and B (Fig. 6) with its 5,504 trees all of which are ICS 1 is very striking, especially as it is adjacent to and south of so heavily infected a field as 22B. The disease-free record of field 23A and B (Fig. 6) although so close to and west of 22B might also be due to the fact that 89% of its 1,813 trees consist of the resistant clones ICS 1, 6, 8 and 95, only 11% being ICS 60 which appears to be not highly susceptible.

Table II summarises the records of disease incidence in certain crosses which occur in two fields with populations of 4,140 and 8,423 trees and incidences of 1.2% and 2.6% respectively. The incidences for these fields are represented in the table by the letters A and B, respectively, which precede the percentage incidence of disease in the given crosses. The figures in brackets are the original numbers of trees of each cross.

These figures indicate that crosses with ICS 45, 54 or 101 as a parent are very susceptible, those with ICS 1 parentage are suspect, while those with ICS 95 and ICS 98 parentage are more resistant.

Further observations

These observations have been restricted to a single locality and although they might provide useful information, the interpretation of this has certain limitations. In South America the disease occurs in the drier cacao growing areas and it has been suggested that the outbreak in Trinidad was induced by the weakened condition of the island's cacao caused by two very dry years in succession. Table

TABLE I

Incidence of disease in clones as at 31st December, 1958.

Clone	Field									
	7		9C		6A		19		22B	
	Original No. of trees	Percentage diseased	Original No. of trees	Percentage diseased	Original No. of trees	Percentage diseased	Original No. of trees	Percentage diseased	Original No. of trees	Percentage diseased
ICS 1	172	0.0	30	0.0	480	0.2	99	0.0	384	0.0
ICS 6	30	0.0	30	0.0	—	—	100	0.0	—	—
ICS 8	24	0.0	30	0.0	—	—	99	0.0	—	—
ICS 16	24	0.0	30	0.0	—	—	—	—	—	—
ICS 38	30	23.3	—	—	96	5.2	—	—	—	—
ICS 39	85	2.4	30	0.0	192	1.6	80	3.8	—	—
ICS 40	30	0.0	30	0.0	96	0.0	—	—	—	—
ICS 44	30	6.6	30	0.0	—	—	—	—	—	—
ICS 45	30	3.3	30	3.3	—	—	—	—	384	13.5
ICS 60	211	3.3	30	0.0	—	—	—	—	448	4.0
ICS 89	24	0.0	30	0.0	—	—	80	0.0	—	—
ICS 95	241	0.4	30	0.0	—	—	639	0.9	—	—
ICS 98	30	0.0	30	0.0	—	—	80	0.0	—	—
IMC 57	30	0.0	—	—	—	—	—	—	—	—
IMC 67	30	0.0	—	—	—	—	—	—	—	—
SCA 6	—	—	—	—	96	0.0	—	—	—	—
SCA 12	—	—	—	—	96	0.0	—	—	—	—

III shows that the rainfall at River Estate for the period June, 1956, to May, 1958, was distinctly lower than the average for the ten-year period 1949 to 1958.

TABLE III

River Estate rainfall in inches

Year	Dry season	Wet season	Total
	January to May	June to December	
1956	21.42	46.20	67.62
1957	11.88	53.79	65.67
1958	13.90	63.72	77.62
1949 to 1958 average	19.26	60.68	79.94

River Estate is among the drier cacao growing areas of Trinidad and it might be profitable to study the disease in a wetter area of the island. Meanwhile further observations are being made at River Estate. Much more needs to be known of the bionomics of the fungus and the insects associated with this disease. It would appear that until the role of the *Xyleborus* beetles is fully determined no rational approach could be made to the problems of controlling this disease.

Control

At present both sanitation and chemical protection methods are being tried at River Estate. The dead trees are being dug out and burnt and the remaining trees are being sprayed with insecticide. Gammexane dispersible powder is being applied in low volume by mist blower. The choice of this BHC product is based on reports of its success in controlling *Xyleborus* borers in felled timber in other tropical countries. The aim is to save the field experiments from ruin rather than to experiment with insecticides.

Summary

The symptoms of a wilt disease in cacao due to *Ceratostomella fimbriata* at River Estate are described and evidence indicating its transmission by wind and insects is discussed. Tentative conclusions on the relative susceptibility of various cacao clones and crosses are put forward. The empirical methods of control now in use are mentioned.

Acknowledgments

All the names cannot be recorded here, but the writer wishes to thank all who helped in this work, directly or indirectly. To the Caribbean Commission thanks are due for translations and reprints. To Mr. G. Stell, Entomologist, Department of Agriculture, Trinidad and Tobago, the writer is grateful for identification of the insects. Special thanks go to Dr. G. Maliphant, Soils Department, for releasing his field assistants for the survey, to Mr. V. White, Assistant Manager, River Estate, for very helpful information and observations, to Mr. D. Murray

TABLE II

Incidence of disease in crosses as at 31st December, 1958

Parent ♀	♂ ICS 1	ICS 6	ICS 8	ICS 12	ICS 16	ICS 38	ICS 39	ICS 40	ICS 45	ICS 54	ICS 60	ICS 89	ICS 95	ICS 98	ICS 101	M 8	Open pollinated
ICS 1	B 0.8 (236)	B 5.2 (136)	B 5.6 (215)	—	B 0.4 (253)	—	A 0.0 (100)	A 0.0 (100)	B 5.4 (148)	—	B 1.2 (256)	A 1.0 (100)	A 0.0 (100)	A 0.0 (100)	—	B 2.8 (143)	—
ICS 6	B 0.7 (138)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
ICS 8	—	—	—	—	—	—	—	—	—	—	—	—	A 0.0 (100)	A 0.0 (100)	—	—	—
ICS 12	—	—	—	—	—	—	—	—	B 9.5 (169)	B 7.8 (179)	—	—	—	—	B 15.8 (139)	—	—
ICS 16	B 0.0 (206)	—	—	—	—	—	—	—	A 1.0 (100)	—	—	—	—	—	—	—	—
ICS 38	—	—	—	—	—	—	—	—	A 13.0 (100)	—	—	—	—	—	—	—	—
ICS 39	B 6.6 (60)	B 0.0 (80)	A 2.0 (100)	—	—	—	—	—	—	—	—	—	A 1.0 (100)	—	—	—	B 5.6 (88)
ICS 40	—	A 0.0 (100)	A 1.0 (100)	—	—	—	—	—	A 1.0 (100)	—	—	—	—	A 0.0 (100)	—	—	—
ICS 45	B 3.2 (123)	—	—	—	—	—	A 9.0 (100)	—	B 3.3 (151)	B 10.8 (167)	B 2.45 (212)	—	A 1.0 (100)	A 1.0 (100)	B 19.2 (156)	B 0.0 (96)	—
ICS 54	—	—	—	—	—	—	—	—	—	—	—	—	—	—	B 12.7 (141)	—	—
ICS 60	B 1.3 (320)	—	A 1.0 (100)	—	—	—	—	—	B 5.8 (208)	—	—	—	A 1.0 (100)	A 0.0 (100)	—	—	—
ICS 89	B 4.9 (122)	B 0.0 (64)	—	—	—	—	—	—	A 8.0 (100)	—	—	—	A 1.0 (100)	—	—	—	B 2.2 (90)
ICS 95	B 2.3 (88)	B 1.2 (80)	—	—	A 1.0 (100)	—	—	A 2.0 (100)	—	—	—	—	A 1.0 (100)	—	—	—	B 0.0. (80)
ICS 98	B 3.7 (80)	B 0.0 (80)	—	—	A 1.0 (100)	—	A 0.0 (100)	—	—	—	—	A 0.0 (100)	A 1.0 (100)	A 1.0 (100)	—	—	B 0.0 (80)
ICS 101	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
M 8	B 1.6 (185)	—	—	—	—	—	—	—	B 1.0 (96)	—	—	—	—	—	—	B 0.4 (225)	—

for discussions on various aspects of the problem, to Dr. B. Bartley both for his translations of Spanish papers and his discussions and to Samaroo Mahabir for the photography.

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Heat treatment of cacao budwood with reference to cacao virus

by

D. B. Murray and J. T. Swarbrick

ALTHOUGH neither of the two strains of cacao virus present in Trinidad appears to be lethal the possibility always exists of a more virulent form arising by mutation. The policy adopted by the Trinidad Department of Agriculture and the Cocoa Board has been to confine the virus to the Northern range area, where it was first discovered, by the maintenance of a *cordon sanitaire*. Eradication by cutting out within the virus area was not practicable and so far there has been no report of its spread to any new area outside the *cordon sanitaire*.

It so happens, however, that River Estate lies within the virus area and virus is present in certain fields in spite of attempts to eradicate it by cutting out infected trees. The possibility is, therefore, always present that new planting material produced in the breeding programme might become infected and that potential high yielding trees be rendered valueless.

It is possible to inactivate some viruses in vegetative plant material by the use of heat treatments and Posnette (1944) tried the effect of hot water treatment on cacao budwood infected with Trinidad strain A. At a temperature of 50°C., the material remained viable with immersions of up to 8 minutes. After a 10 minute immersion most of the material died. There was no inactivation of the virus. Longer immersion at a lower temperature, i.e. 45 minutes at 43.4°C. also proved ineffective. Similar work at the West African Cocoa Research Institute (Thresh, 1958) showed that inactivation of the virus from the New Juaben and Bisa area of Ghana did not occur when budwood was treated at 45°C. for thirty minutes, at 50°C. for 12 minutes or at 52°C. for 10 minutes. More drastic treatments killed the budwood.

In view of the special circumstances involved at River Estate a further attempt has been made on the problem using Trinidad strain B as the material for study.

Experimental results

As a preliminary, the upper thermal death point of healthy cacao budwood from the clone ICS 95 was investigated. After treatment in a thermostatically controlled hot water tank the material was patch budded on to seedling stocks and examined after 10 days. The results of the last three trials only are given in Table I.

These results indicate a slightly greater tolerance for healthy budwood than found by Thresh for infected material. The trials on infected material (Table II) showed, however, that infected material does have a rather lower tolerance than healthy and the thermal death points for budwood infected with strain B approximated to those found by Thresh.

TABLE I

Survival of healthy buds subjected to treatments at various temperatures for varying periods

Trial No. 7

Temp.	Mins.	Live buds	Dead buds
45°C.	10	5	1
47.5°C.	10	6	0
	20	6	0
	30	4	2
50°C.	5	3	3
	10	5	1
	15	2	2
52.5°C.	5	3	1
Control	—	9	5

Trial No. 8

Temp.	Mins.	Live buds	Dead buds
47.5°C.	40	6	0
50°C.	20	6	0
	25	5	1
52.5°C.	10	5	1
	15	5	1

Trial No. 9

Temp.	Mins.	Live buds	Dead buds
50°C.	25	5	1
	30	3	3
	35	1	5
52.5°C.	15	5	1
	20	2	4
	25	0	6

TABLE II

Survival of virus infected material subjected to treatments at various temperatures for varying periods

Trial No. 12

Temp.	Mins.	Live buds	Dead buds
47.5°C.	40	6	0
50°C.	10	6	0
	15	6	0
52.5°C.	5	5	1
	10	0	6

Trial No. 13

Temp.	Mins.	Live buds	Dead buds
50°C.	15	6	0
	20	0	4
	25	2	4
52.5°C.	5	4	2
	8	2	2

All buds that survived showed virus symptoms in their first growth flush, none of the treatments proving effective in inactivating the virus. It must, therefore, be concluded that heat inactivation of cacao virus is not possible and every effort made to insure that material bred at River Estate does not become infected.

As a side line to this work the lower thermal death point of budwood was also investigated. This is of some interest in the carriage of budwood by air. It was not possible with the equipment available to achieve and maintain temperatures lower than 3°C. and at this temperature exposures of up to 40 minutes did not lead to loss of viability : an interesting observation, moreover, was made to the effect that time to bud break was, apparently, considerably reduced by the cold water treatment. It has not yet proved possible to explore this line which might have a useful practical application.

Summary

(1) In view of the possibility of cacao bred at River Estate becoming infected by virus and thus rendered valueless, attempts have been made to inactivate Trinidad virus strain B by various hot water treatments.

(2) Healthy budwood has a slightly higher heat tolerance than infected, but no inactivation of the virus occurred with treatments at the limits of heat tolerance.

(3) Cold water treatments, i.e. up to 40 minutes at 3°C. seem to accelerate bud break of patch buds of cacao.

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Effects of delayed filling of cacao fermentation boxes

by

D. B. Murray and L. A. Griffiths

THE traditional fermentation process for Trinidad cacao involves putting the freshly harvested beans into wooden "sweat" boxes which are covered down with banana leaves and possibly a wooden cover. The contents are turned into another similar box after 2 days and this turning is repeated until the fermentation is considered complete at the end of 6 to 8 days.

Many of the Trinidad estates have very large sweat boxes, which may have a capacity of four to eight thousand pounds of wet cacao. When the estates were highly productive it was possible to harvest this quantity of cacao and fill a sweat box in one day. Or, alternatively, the harvested pods were left in the field and broken open after two or three days when a sufficient number had been accumulated to fill a box. This practice gave the high quality Trinidad Plantation cocoa with its characteristic quality and flavour.

For many years now these practices have not obtained. Production is seldom sufficient to fill a large sweat box in one day and praedial larceny is so prevalent that leaving harvested pods in the field would be inviting their loss. The procedure, therefore, has developed of harvesting and extracting the beans each day and of adding these small quantities of beans to a sweat box until it is full or sufficiently full to be considered adequate for starting the fermentation. It is then covered down with banana leaves and this is considered as the day of starting the fermentation. Anything up to a week may have elapsed between the first and final additions to the box.

The deterioration in the quality of Trinidad cocoa has been a source of complaint for a long time. The two factors on which the blame has been laid are, firstly, the inherent flavour quality or lack of quality, of the ICS clones used in the rehabilitation programme and, secondly, poor and unsatisfactory fermentation and drying.

As regards the first, the proportion of clones on most estates is still small in comparison with the old seedling cacao. Certain clones especially ICS 1 are known to be weak in chocolate intensity, but this clone only represents a small proportion of the total distributed, others such as ICS 95 giving a stronger chocolate intensity. No single clone gives the traditional Trinidad flavour, but this point will not be discussed here. The point at issue is the factor or factors responsible for the general deterioration in quality and the evidence suggests that this is mainly a result of the modifications in fermentation practice. Though this view would probably be fairly widely accepted no experimental work has been undertaken to show the degree of deterioration. Accordingly an experiment has been carried out to determine to what extent the practice of filling a sweat box over several days affects the final product.

Experimental details

The trial was carried out at River Estate using four similar sweat boxes. As harvesting and filling were to be spread over a period of 6 days the complication of using different mixtures at different times was resolved by using a mixture of three parts of ICS 1 and one part of ICS 95 for each addition to the sweat box. ICS 1 was not a happy choice, not only on account of its weak chocolate flavour, but on account of the varying proportion of pale criollo type beans it carries. It was, however, the only clone available in sufficient quantity.

The total charge for each sweat box was 760 lb., which was considered about the minimum to give a satisfactory commercial fermentation.

The procedure for filling the four boxes was :—

Box 1. Day 0—Completely filled.

„ 2. Day 0—$\frac{1}{2}$ filled, Day 2—completed.

„ 3. Day 0—$\frac{1}{3}$ filled, Day 2—$\frac{1}{3}$ filled, Day 4—completed.

„ 4. Day 0—$\frac{1}{4}$ filled, Day 2—$\frac{1}{4}$ filled, Day 4—$\frac{1}{4}$ filled, Day 6—completed.

The cacao from each box was turned into another box every other day according to normal practice. On Day 8 samples were drawn from each box and sun dried. Of these samples only that from Box 1 would have had a full 8 days fermentation. The last entry to Box 4 would have had only 2 days.

Further samples were drawn every 2 days until the last entry to Box 4 had had 8 days fermentation. The first entry would have been in the box for 14 days.

The samples, 10 in number, were, therefore, made up as follows :—

Sample No.	Proportion	Days of fermentation
1	All	8
2	Half each	8, 6
3	One-third each	8, 6, 4
4	One-quarter each	8, 8, 4, 2
5	As 2	10, 8
6	As 3	10, 8, 6
7	As 4	10, 8, 6, 4
8	As 3	12, 10, 8
9	As 4	12, 10, 8, 6
10	As 4	14, 12, 10, 8

It is obvious that the extremes of conditions are found in sample 4 where $\frac{1}{4}$ of the bean have had only 2 days fermentation and in sample 10 where $\frac{1}{4}$ have had 14 days.

In general external appearance there was little difference between any of the samples. Having been sun dried in small quantities they lacked the external polish developed in commercial batches by the practice of dancing. When the beans were cut, however, it was obvious that very marked differences existed between the samples in the percentage of well fermented and under-fermented beans.

With the co-operation of Dr. C. Y. Shephard and the Cocoa Planters Association of Trinidad a commercial assessment of these samples was made in which 9 examiners from cocoa exporting firms ranked the samples. The average of their results constitutes Table I.

TABLE I

% beans in each class

Sample No.	Well fermented	Under fermented	Cheesy	Rank
1	81	18	1	1st
2	60	35	5	2nd
3	50	33	17	5th
4	25	26	49	9th
5	54	30	16	4th
6	36	34	30	7th
7	17	24	60	10th
8	61	29	10	3rd
9	48	20	32	6th
10	31	26	43	8th

In the classification less attention was given to colour and emphasis laid on physical character of the break. Well fermented beans had a complete " break " and a bright lively appearance, under-fermented had some break, and the very under-fermented were cheesy with no break at all presenting an entire or completely smooth surface.

Agreement between examiners was good and all ranked No. 1 as the best sample. This sample was not perfect, containing as it did some 18% under-fermented beans. The degree of under-fermentation was, however, small in comparison with all the remaining samples.

The comparison should first be made between samples Nos. 1, 5, 8 and 10 which would represent the results of estate practice. Though there is little difference between No. 5 and No. 8—sampling variation was not determined—they are both decidedly poorer than No. 1 especially in regard to cheesy beans. No. 10 from the box filled over 6 days is by far the worst of the four.

Nos. 4, 7, 9 10 where 6 days were taken to fill the box were uniformly bad ; the longer fermentation period doing little to improve the quality. In the last of these a proportion of over-fermented beans would be expected, but these proved difficult to pick out and no separate classification was possible.

Tasting tests

All samples were made into chocolate by the standard procedure and tasted by the I.C.T.A. panel. Since it is not possible to taste a large number of samples at one sitting the samples were sent out in batches as follows :—

1. Samples 1, 2, 3, 4.
2. ,, 1, 2, 5.
3. ,, 1, 3, 6, 8.
4. ,, 1, 4, 7, 9, 10.

Each sample was assessed in comparison with No. 1 for chocolate intensity, acidity, bitterness and astringency, fruity and off flavours.

It is not intended to reproduce the scoring in detail, but only to indicate the general trends. No sample was rated as high as No. 1 for general chocolate intensity and all showed a higher degree of bitterness and astringency. No. 2 ranking second would seem to indicate that a 6-day fermentation would be adequate and this is probably due in no small measure to the high proportion of pale ICS 1 beans, which are known to require a shorter than average period of fermentation.

Those samples particularly Nos. 9 and 10 in which a proportion of the beans had been in the box for 12 or 14 days developed the off flavours associated with protein breakdown and were extremely poor. None of the other samples were as good as Nos. 1 and 2.

Discussion

These results confirm the generally accepted impression that delay in completing the filling of the sweat box is a bad practice. The extent of the deterioration is, however, unexpectedly marked both in regard to the visual appearance of the cut bean and in the flavour of the bean.

The reasons for the loss in quality must be complex. Firstly, in a small volume of beans in a box, fermentation will start, but the higher surface to volume ratio will result in an appreciable heat loss. The addition of a second batch of cold beans will also serve to keep temperature down. It was, unfortunately, not possible in this experiment to record fermentation temperature in each treatment.

Again, the micro flora associated with a normal fermentation and the changes in its composition, i.e. yeasts giving way to Acetobacter will operate under different conditions of sugar concentrations, temperatures, etc. in a mixture of beans placed in the box at varying times. These changes would need to be worked out in more detail in small scale experiments since it would not be feasible to run a series of experiment like the one described requiring as it does over 3,000 lb. wet cacao.

The practical results are clear cut—any departure from filling a sweat box and starting the fermentation in one day leads to a marked deterioration in the final product. The longer the period for filling the box the worse the result and prolonging the period of fermentation will not correct the original trouble.

There are several alternatives facing the grower. He can make his sweat boxes smaller and this can be done with existing boxes by sub-divisions with removable partitions. Even the estate with a large production will face periods at the beginning and end of the season when only a small quantity of cocoa is coming in.

The minimum size of box has not been established experimentally, but probably lies at about 2 to 2½ ft. cubed. This would hold from 400 to 800 lb. wet cocoa. Alternatively, the pods can be brought in from the field until a sufficient quantity have accumulated to fill a box. This involves additional transport and would not find favour with the planter. Finally, and more particularly

for the small man improvement lies in the development of co-operative fermentaries where the small quantities of wet beans brought in can be bulked to permit the filling of a standard sweat box in a single day.

Acknowledgment

The writers are indebted to Dr. C. Y. Shephard* for the great interest and assistance given in this study.

* Dr. C. Y. Shephard died on 16th June, 1959.

Summary

(1) The effect on bean appearance, quality and flavour of prolonging the period over which a fermentation box is filled has been investigated.

(2) Any departure from the practice of filling the box in one day leads to a deterioration in all these factors.

On the Optimal Conditions for Microfermentation: A Critical Review

by

L. A. Griffiths

(i)

In view of the many conflicting statements in the literature relating to the optimal conditions for microfermentation, it appeared desirable to examine in some detail the experimental basis on which these claims rested. Particular attention has been given to the procedures described by Wadsworth and Howat (1954) and Forsyth and Quesnel for the small scale fermentation of cacao. Both of these procedures are non-microbial methods, which are claimed to give a good quality cocoa. In the procedure of Wadsworth and Howat (1954), the sterile unpulped beans are incubated at two successive temperature levels (37° and 50°C.) over a period of 7 days and the CO_2, evolved, removed by absorption in KOH. In the method of Quesnel (1957), washed beans are incubated in a 1% acetic acid/1% ethanol aqueous solution for 48 hours at 47°C.

(ii) Requirement for acetic acid

Although acetic acid is formed in considerable amounts in the course of sweat-box fermentations, opinions vary considerably on the need for this acid in laboratory fermentation procedures. The experimental fermentations of Wadsworth and Howat (1954) which were conducted under apparently aseptic conditions, were reported to give rise to cacao possessing good chocolate flavour. Since this observation suggested that acetic acid was not essential to the development of chocolate flavour, as acetic acid is not a normal metabolite of bean enzymic activity, the experiments were repeated under carefully controlled conditions of asepsis. Sterile bean material was obtained by dissection of ripe pods employing sterile instruments in a specially constructed glass-fronted aseptic cabinet. The use of the antibacterial preparation, Vantoc B, was thus rendered unnecessary. After filling previously prepared sterile vessels according to the procedure laid down by Wadsworth and Howat, the fermentation was allowed to proceed under the prescribed conditions.

A check on asepsis was made by withdrawing "fermenting" material by means of a sterile needle and transferring it to sterile, enriched, agar plates. The absence of colonies on the agar indicated the success of the aseptic technique. After fermentation the beans were dried and made into chocolate by the standard C.S.A.C. procedure. In all cases it was shown that where asepsis had been successfully maintained throughout fermentation the resulting beans were low in chocolate flavour and, moreover, the only bean material prepared by this technique which possessed moderate chocolate flavour was material which had been accidently contaminated. A further series of experiments, in which the effect of a series of dilutions of Vantoc B, on the growth of cacao microflora in pulp liquors, was examined and indicated that at the concentration employed by Wadsworth and Howat, vigorous growth of certain members of the microflora, including the Acetobacter, occurred. It is evident, therefore, that the possibility of Acetobacter being present in the fermentations conducted by Wadsworth and Howat cannot be excluded and, furthermore, the experiments described above do not lend support to the view that acetic acid is not essential for the formation of chocolate flavour.

The requirement for acetic acid was further studied employing the microfermentation technique of Quesnel (1957). This method was observed in this laboratory to give rise to bean material, possessing moderate chocolate flavour. The procedure consists essentially of incubating washed beans in 1% acetic acid/1% ethanol at a temperature of 47°C. for 48 hours. In a series of experiments, the effect of replacing the acetic acid/ethanol solution with other aqueous solutions was studied. When the beans were incubated in water alone, the chocolate intensity was very low and the dominant flavour was one of marked astringency. With the additions of 1% alcohol there was some reduction in astringency, the dominant flavour being of a sweet "fatty" character. The substitution of acetic acid by a range of other organic acids, including salicylic, benzoic, citric, malic, tartaric and succinic acids did not give rise to any appreciable degree of chocolate flavour. A large range of other classes of compound was investigated as lytic agents, including mineral acids, sugars and sugar alcohols, but in no case was an increase in chocolate flavour observed above that of the water control, which in all cases remained extremely low. It was concluded from these observations that the presence of acetic acid was essential for the production of chocolate flavour.

(iii) Requirement for aeration

The fermentation process has been divided by Forsyth (1957) into an "Anaerobic Hydrolytic Phase" and an "Oxidative Condensation Phase" which occur successively. The anaerobic phase is held by him to be essential and it is claimed that omission of this stage leads to beans completely lacking in flavour. In contrast with this, it has been stated by Powell (1957) that in his fermentation experiments limited aeration proved beneficial to flavour development and Maclean (1950) reported that restricted aeration improved flavour development. Rohan (1958)

has claimed that an improvement in quality and a shortening of fermentation time results when the aeration of fermenting cacao is increased by fermentation in shallow layers.

It is important in considering those claims to recognise that an oxygen effect may operate at more than one locus. Firstly, it is evident that in microbial fermentations, which are dependent upon a vigorous growth of the aerobic Acetobacter, the maintenance of completely anaerobic conditions indefinitely would lead to a suppression of Acetobacter development, reduction of the acetic acid content of the pulp and a decline in flavour (cf. section ii). There seems to be little doubt that the beneficial effects of aeration observed by Rohan (1958) and Maclean (1950) were the result of a stimulus to Acetobacter development. Although an attempt was made in the large scale fermentation of Howat *et al.* (1958) to maintain asepsis during the fermentation process, it appears from the measures adopted to achieve this and from our own observations (section ii) that the fermentations were essentially of a modified microbial type and it is probable, therefore, that the improvements obtained by aeration were indirect and mediated by the microflora.

Since the non-microbial method of Quesnel (1957) had been found in this laboratory to give rise to bean material showing moderate chocolate flavour, the procedure was employed to explore a possible requirement for oxygen in the " fermenting " cotyledons. Firstly, it was shown by a modification in the apparatus of Quesnel that the total exclusion of the air pocket from the fermentation vessels did not impair the resulting chocolate flavour of the product and later by controlled aeration from a cylinder of oxygen, it was shown that an actual decrease in flavour occurred, when the oxygen tension of the suspending medium was appreciably increased. From these observations it follows (*a*) that the formation of the chocolate precursor is an essentially anaerobic reaction and (*b*) that the presence of oxygen is deleterious to flavour development at this stage.

The process by which higher oxygen tensions adversely affects flavour development is not yet clear, although the inhibitory affect of polyphenolic oxidation products on certain of the enzyme systems of the bean noted by Forsyth and Quesnel (1957) suggests a mechanism for this effect.

It has been shown (Griffiths 1958), that the major polyphenoloxidase substrate of cacao is (—) epicatechin ; the leucocyanidins being largely resistant to oxidation and contributing to the formation of the brown oxidation products to a very limited extent. The formation of large amounts of oxidised (—) epicatechin polymers would thus be expected to retard greatly the enzymic reactions occurring in the cotyledon.

A further point requiring elucidation is how oxidation of the cotyledon is suppressed during the earlier stages of fermentation. In sweat boxes of the conventional type, aeration is considerably restricted by the sides and flooring and it is evident that the amount of gaseous and dissolved oxygen reaching the interior of the fermenting mass will initially be small. However, it has been reported by Rohan (1958) that relatively small quantities of cacao can be successfully fermented in short periods of time by considerably increasing aeration of the mass by an increase in the surface area/volume relationship. In recent experiments in Trinidad, in which this claim is receiving intensive study, it was observed that although aeration was greatly increased, resulting in the high initial temperatures reported by Rohan, no marked oxidative browning of the cotyledon occurred before the third day.

This suppression of oxidase activity is attributable to three factors :—

(*a*) The citric acid content of the pulp. Chromatographic studies have indicated the presence of citric acid in the pulp of a wide range of clonal cacaos and it has been shown that cacao polyphenol oxidase activity in dilute aqueous solution is completely suppressed at citric acid concentrations higher than 0.2%. The degree of inhibition was shown to be a function of the pH, which in the pulp liquors of clonal cacaos ranges from a pH of 3.5 for ICS 1 to a pH of 4.45 for SCA 6.

(*b*) Ascorbic acid content of pulp. The presence of considerable amounts of Vitamin C in fresh pulp has been shown by dichlorophenol indophenol titration. The mean value of a wide range of pulps examined was 25.05 mg/100 ml. fresh pulp liquor. The presence of ascorbic acid at a high level in the pulp of *T. cacao* is of interest in view of the observations of Bauernfeind (1953), who showed that enzymic browning in a range of foodstuffs, did not begin until the vitamin C present in the tissues had been destroyed. It is evident, therefore, that the ascorbic acid of cacao pulp will initially stabilise the polyphenols released from the cotyledons of the bean until breakdown of the former substance has occurred.

(*c*) Localisation of polyphenol oxidase in specialised cells. It was earlier reported by Brown (1954) that by differential sedimentation of cell material of the cotyledons of *T. cacao*, it could be shown that the polyphenols are situated in separate cells to those in which the enzymes active towards the polyphenols are contained. By histological studies on sections of cacao cotyledon the above findings have been confirmed and by suspending living sections in aqueous solutions of l-epicatechin, it has been shown that cacao polyphenoloxidase is largely distributed in islets of colourless cells throughout the cotyledon and is also present in the layer of cells investing the fissures contained in the mature cotyledon.

From these observations it is evident (i) that in microfermentations where the pulp has been retained, the bean material will be less susceptible to loss of flavour by early oxidative action. (ii) that in microfermentation procedures employing de-pulped or washed beans, it is essential to exclude air to avoid a deterioration in flavour ; (iii) that cell lysis under conditions of high oxygen tensions is to be avoided.

Role of polyphenoloxidase in flavour development

Whilst it is generally believed that polyphenoloxidase activity reduces the astringency of the fermented beans the evidence for this is largely of an indirect character. Although experiments have been carried out in which inactivation of the polyphenoloxidase was effected, the inactivation was by the application of heat or chemical treatment, which would, in addition to inactivating the polyphenoloxidase, bring about other reactions in the cotyledon, including protein tanning, etc. It appeared desirable, therefore, to employ a technique, which would

suppress polyphenoloxidase activity without causing the polyphenols of the cotyledon to react with other bean constituents.

Since it was shown (section iii) by means of the Quesnel procedure, that the initial stages of fermentation can be carried out under strict anaerobiosis, the problem reduced itself to that of devising a means whereby a total suppression of polyphenoloxidase activity during drying could be maintained. The technique adopted was that of freeze-drying by means of which, water could be removed under negative pressure from the cotyledon at a temperature of 0°C., at which temperature, enzymic activity is completely arrested.

Beans fermented by the modified Quesnel apparatus in which all traces of oxygen were eliminated, were after rapid cooling in a solid CO_2/acetone mixture transferred to vessels connected to a freeze drying unit, and the moisture content reduced to 8% over a period of $7\frac{1}{2}$ hours. The beans on removal from the freeze-dryer were pale-purple in colour and showed no evidence of browning either at the surface or internally. On roasting the beans gave a moderate chocolate aroma and although the astringency was high, chocolate flavour could be detected.

It is evident, therefore, that polyphenoloxidase activity is not essential for the development of aroma and basic chocolate flavour, and that its role in fermentation is restricted to the reduction of astringency. It may also be concluded that no other oxidases participate in the formation of the aroma principle.

Aeration and mould development

It has been observed that a high initial oxygen tension in laboratory-scale fermentations is highly conducive to the development of moulds, which give rise to disagreeable off-flavours in the product. It is, therefore, essential in non-sterile laboratory fermentations to maintain a high degree of anaerobiosis for the first 40 hours. At the end of this period, it was found that restricted aeration could be safely carried out to encourage Acetobacter development without stimulating mould growth. Since the cacao moulds are highly aerobic, suppression of growth by anaerobiosis is easily understood; the failure of the moulds to establish themselves after an interval of 40 hours is presumably due to changes unfavourable to their development, having occurred in the substrate.

(iv) Temperature requirement

The importance of raising the temperature of fermenting beans to the range 45°-51°C. during the later stages of fermentation has been recognised by all leading workers in the field of raw cacao technology. Both laboratory-scale and commercial fermentations, in which the temperature remains low give rise to cacao, in which astringency is high and chocolate flavour low.

It has been suggested by Wadsworth and Howat (1954) that two successive temperature levels are necessary for the formation of chocolate flavour precursor. The earlier level of 35°C. for $3\frac{1}{2}$ days being necessary before the mass is raised to 50°C. for a period of 3 days. The lower temperature phase is held to be necessary to permit "germination processes" to occur. Since it is possible, however, to ferment beans, which have been killed by various physical treatments, by the Quesnel procedure (section vi), the necessity of "germination processes" may be questioned.

In microfermentation procedures dependent upon the endogenous formation of acetic acid, it is evident that low temperature incubation will be necessary for the optimal development of yeasts and Acetobacter required for the formation of acetic acid from the sugar of the pulp. Thus in any microbial or modified microbial fermentation, preincubation at lower temperatures will be beneficial to the course of the fermentation.

(v) Requirement for CO_2 removal

It has been stated by Howat *et al* (1954) that removal of carbon dioxide from small scale fermentation leads to an increase in chocolate flavour. It is important to consider this effect in relation to both the enzymic changes proceeding in the cotyledon and to the microflora. Since it has been shown (section iv), that the earlier phase of fermentation is essentially anaerobic, the adverse effect of CO_2 on fermentation cannot be due to the exclusion of oxygen, although the possibility of a direct influence on the flavour producing reaction(s) cannot be dismissed. In relation to the microflora, it is undoubtedly true, that a high tension of CO_2 would considerably restrict the development of Acetobacter, which are highly aerobic organisms. In the author's opinion, the increase in chocolate flavour obtained in the experiments of Howat *et al.* following CO_2 removal is attributable to stimulation of the growth of the Acetobacter population, which would have been reduced, but not eliminated by the microbiological control technique employed.

(vi) Requirement for germination changes before death of bean

Although it was earlier reported by Howat *et al.* (1954) that the development of chocolate flavour is dependent upon germination changes preceding the death of the bean, the need for such changes has been questioned by Quesnel (1957). Experiments in this laboratory employing the microfermentation method of Quesnel indicated that beans previously killed by cooling to 4°C. and by heating at 55°C. gave rise, as reported by Quesnel, to chocolate equivalent to the controls in flavour. It was, however, observed, in contrast with the observations of Quesnel, that in the case of bean killed by sun drying the chocolate flavour was appreciably weaker than in the controls. It is believed that physical changes in the tissue of the cotyledon during drying were responsible for this decrease in flavour since dried beans were appreciably less permeable to the aqueous acid solution and entry of acetic acid into the tissue was considerably delayed.

(vii) Maintenance of humidity

Special precautions must be adopted in laboratory-scale fermentations to maintain a high humidity level in the fermenting vessel. The use of cotton wool plugs should be avoided since the resulting drying out of the surface layer retards the essential chemical changes in the beans and increases the astringency of the sample.

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A Laboratory Procedure for Micro-fermentation and Small-scale Drying

by

L. A. Griffiths

On the basis of the observations described in the preceding paper (Griffiths 1959), a method has been developed for the fermentation of small quantities of cacao. Although experiments indicated that a sterile cacao " fermentation " was possible, if the major metabolic products of the micro-organisms, viz. ethanol and acetic acid were supplied preformed as in the method of Quesnel (1957), the product was found to be inferior to the commercially fermented controls. It was concluded that although the procedure described by Quesnel is a valuable experimental tool, the method cannot be expected to give the wide range of auxillary flavours, which are of importance in Trinidad cacao and are the result of an interaction between the normal microflora of the sweat box and the inherent flavour potential of the bean. Assessment of the Quesnel technique at the Imperial College of Tropical Agriculture indicated that the chocolate possessed a moderate basic chocolate flavour, but that astringency was high and that the auxillary " Trinidad " flavours were low or absent.

An attempt was, therefore, made to develop a small scale method, in which conditions would approximate as closely as possible to the normal course of events in a commercial fermentation. It was felt that such a method would give a more exact forecast of the flavour character of a new cocoa variety, than one in which the conditions of the fermentation differed considerably from those of normal estate practice.

Principle

The physical, environmental conditions are carefully controlled to correspond to sweat box conditions. By means of this control it is possible selectively to promote the development of the normal microflora from a small mixed natural inoculum and to reproduce the normal sequence of organisms observed during the course of a large scale fermentation.

Apparatus and Equipment

Incubators. B.T.L. universal incubators

Fermentation Vessels (Fig. 1). The vessels employed were glass cylinders (capacity 2,500 cc.) with ground glass rims fitted with circular glass plates. Each cylinder contained a perforated wooden plate (diam. 5 in.) supported on a glass dish to raise it to a height of 1½ in. above the bottom of the jar.

Inoculum

The inoculum is obtained from an estate sweat box late on the second day. The microflora at this stage is transitional and samples withdrawn from the box contain both yeasts and Acetobacter.

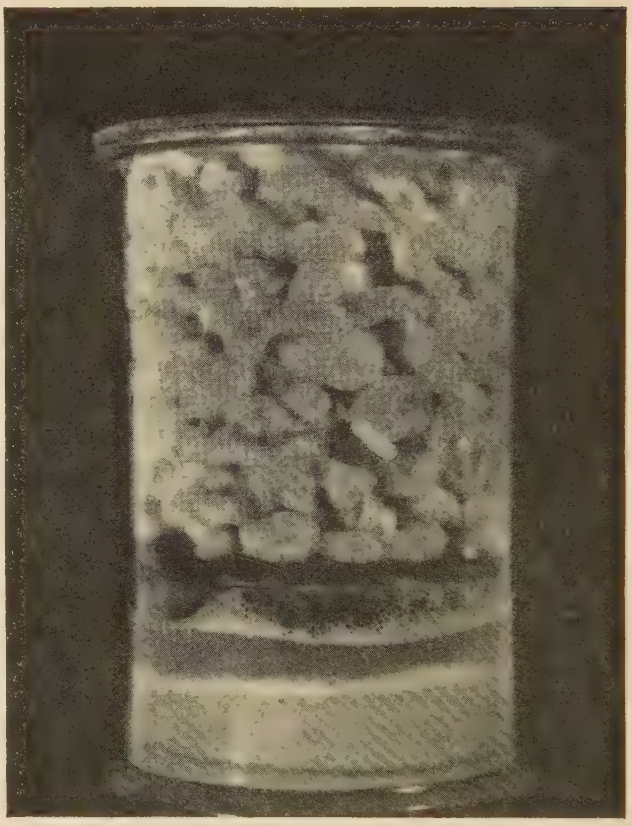

Figure 1

A 50 gm. sample of beans and adhering pulp are shaken with 100 ml. of distilled water and filtered through muslin. The turbid filtrate is then centrifuged at 4,000 r.p.m. for 20 minutes, when the yeasts, bacteria and cell debris are thrown down and the supernatant containing esters, acids and other flavour principles is decanted off and discarded. The residue is washed by re-suspending in 2 × 100 mls. of distilled water and finally suspended in 100 ml. of distilled water for use as an inoculum.

Procedure

Sufficient pods to give *ca* 2 Kg. of wet cacao are opened and the beans transferred to a large glass beaker (capacity 2 l.). The inoculum, suspended in 100 ml. of distilled water, is then well mixed with the cacao. The beans are

poured into the fermentation vessels, which are completely filled to reduce the amount of air present and the lids are placed in position. It is not necessary to grease the ground glass rim, as the small amount of air diffusing between the surfaces is beneficial to the microflora. Care must be taken, however, that the lids are not displaced as increased aeration favours mould growth and drying out of the upper layer will also occur. The beans are incubated at the following temperatures over the time periods shown :—

°C.	*Period*
30°	0-24 hrs.
35°	24-48 ,,
42°	48-72 ,,
49°	72-216 ,,

During the period of incubation the fermenting pulp liquor accumulates at the bottom of the jar below the wooden platform. This reservoir serves to maintain a high humidity throughout the vessel. Towards the end of the second day, aeration, which is beneficial to the development of the Acetobacter may be increased by introducing a strip of waxed paper between the rim of the vessel and the glass lid. Although the aperture is small, diffusion of CO_2 from the vessel and entry of atmospheric oxygen bring about rapid changes in the internal atmosphere.

" *Turning.*" The fermentation vessels are removed from the incubators at 48 hour intervals for " turning." This is carried out by transferring the beans from each jar to a large beaker, in which they are well mixed. If the amount of pulp on the beans was initially small and any degree of drying out is apparent, a small amount of pulp liquor from the lower part of the jar should be poured over the sample and the moistened beans then replaced in the fermentation vessel. " Turning " was found to be as beneficial in laboratory-scale fermentation as in the estate sweat box. The beneficial results of " turning " are attributable to a more uniform distribution of the microflora throughout the bean mass and to the corresponding development of a uniform chemical environment.

Drying of small samples

In our earlier experiments, small bean samples were dried by exposure to the sun's rays in a ventilated glasshouse. It became apparent, however, that this treatment was too severe for samples of small size as the drying was rapid and tended to increase the harshness of the bean. Later experiments, in which drying on the laboratory bench was attempted, indicated that such samples were very prone to develop mould due to the static moisture-laden atmosphere. It was found, however, that mould growth could be completely suppressed by the use of an electric fan (G.E.C. 18 in.), which maintained an air stream of constant velocity over the sample during the drying period. To remove the last traces of excess moisture the beans were exposed to overhead Westinghouse Infra-red heat lamps (250 W.), the air stream from the fan being maintained until the beans were removed to an air-conditioned store room.

The procedure was not fully standardised as it was found that the period of drying necessary for the different types of cacao varied according to the size of the bean and the nature of the pulp. Drying was, accordingly, ended when the beans developed a satisfactory " break."

Assessment

A series of bean samples prepared by this method were made into chocolate by the standard C.S.A.C. method and the flavour assessed by a group at the Imperial College of Tropical Agriculture. The chocolate was found to possess moderate to strong basic chocolate flavour, and the intensity of the auxillary " Trinidad " flavours varied according to the clone or variety. Off flavours due to mould and spore-forming bacilli were wholly absent.

A further series of samples prepared by this technique, was subsequently despatched to Mr. B. L. Hancock, of Messrs. Rowntrees, York, who reported that the series fell within the normal range of Trinidad estate cocoas. Foreign flavours were absent and certain of the samples were described as being of a moderate to heavy type of Trinidad cocoa. It is evident, therefore, that by this technique it is possible to bring about a full development of the inherent flavour of high quality cacao.

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The Nutrition of Cacao. Part II

Leaf nitrogen and phosphorus contents

by

G. K. Maliphant

In a previous publication (Jones, Maliphant and Havord 1957) details of a crop logging procedure for cacao were given. The present paper contains some of the results of leaf analyses on samples taken during the first period of the operation of the procedure, May, 1956-July, 1957. All the results discussed here were obtained from the shade, spacing and fertiliser experiment (Experiment I) (Havord 1953), and are thus from ICS 1 clonal trees in their eighth year, which have been planted on a sandy loam soil of imperfect drainage. It was possible to sample the 64 experimental plots six times during this period, the sampling dates being 4th May, 29th June, 2nd October and 21st November, 1956, and 11th February and 21st May, 1957. Only leaf nitrogen content expressed as N% oven-dry matter (O.D.M.) and leaf phosphorus content expressed as P_2O_5% O.D.M. will be considered.

Leaf nitrogen content

The effect of the time of sampling on leaf nitrogen content is illustrated in Fig. 1, where the mean leaf nitrogen contents from shaded and unshaded unfertilised plots are shown.

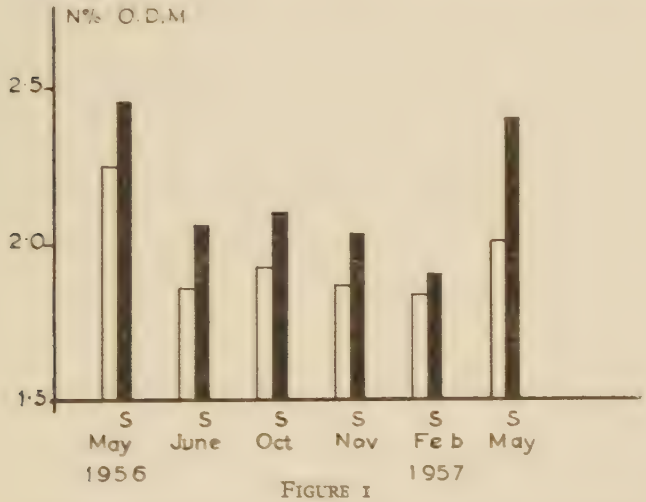

FIGURE 1

Variation in nitrogen content of leaves from unshaded and shaded (S) plots with time of sampling.

Nitrogen fertiliser application and the presence of immortelle shade trees caused an increase in leaf nitrogen content. There was a marked negative interaction between these two factors. The magnitude of these effects varied with the time of sampling, the shade effect being largest in the samples taken at the beginning of the wet season, and the nitrogen effect being largest in samples taken soon after the application of fertilisers (8th May, 1956, and 1st May, 1957). These facts are illustrated in Fig. 2 where the increments of leaf nitrogen content due to the presence of shade and nitrogen fertiliser singly and in combination for the six samples are shown. The variation between duplicates was small and an average least significant difference (P=0.05) for these increments is also indicated.

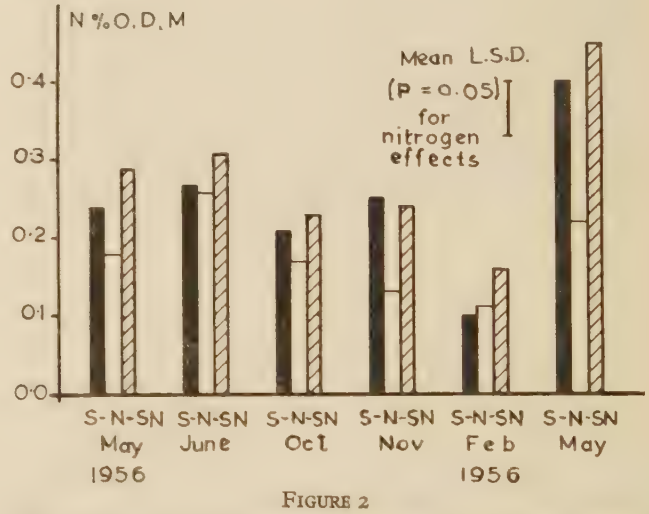

FIGURE 2

Effect of shade (S) and nitrogen fertiliser (N) on leaf nitrogen content.

The effect of nitrogen fertiliser applications was significant at all sampling times, but only in the absence of shade. There was no apparent correlation between leaf nitrogen levels and either rainfall or soil moisture.

Phosphate fertilisers had no marked effect on leaf nitrogen contents.

The effect of potash fertilisers and the interaction with shade is illustrated in Fig. 3.

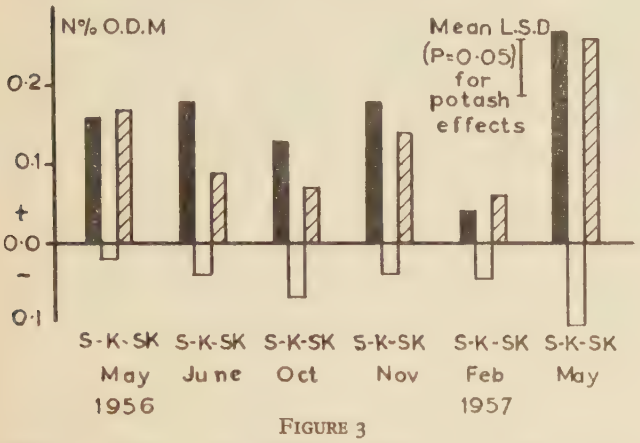

FIGURE 3

Effect of shade (S) and potash fertiliser (K) on leaf nitrogen content.

Potash fertilisers depressed leaf nitrogen contents, significantly so in samples taken immediately after the application of fertilisers. This effect was generally greater in the absence of shade.

Leaf phosphorus content

The effect of time of sampling on the phosphorus content of leaves from unfertilised plots is shown in Fig. 4. The effect of time of sampling was much larger than that shown in Fig. 1 for leaf nitrogen, but the pattern was similar. Again, no correlation with rainfall or soil moisture content was apparent.

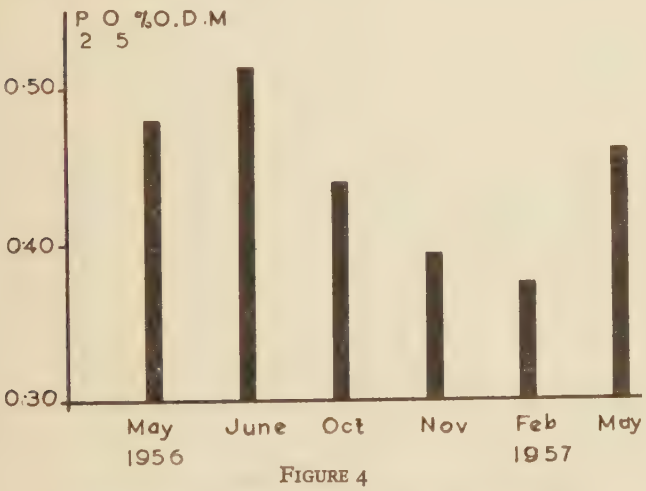

FIGURE 4

Variation in leaf phosphorus content with sampling time.

Phosphate fertiliser applications increased leaf phosphorus contents, this effect being most marked in the absence of shade, and increasing with successive samples. The presence of immortelle shade decreased leaf phosphorus levels, but this effect decreased with successive samples (Fig. 5).

Nitrogen fertiliser applications reduced leaf phosphorus contents (Fig. 6). This effect was much more marked in the absence of shade and was frequently larger than the corresponding increase due to phosphate fertiliser.

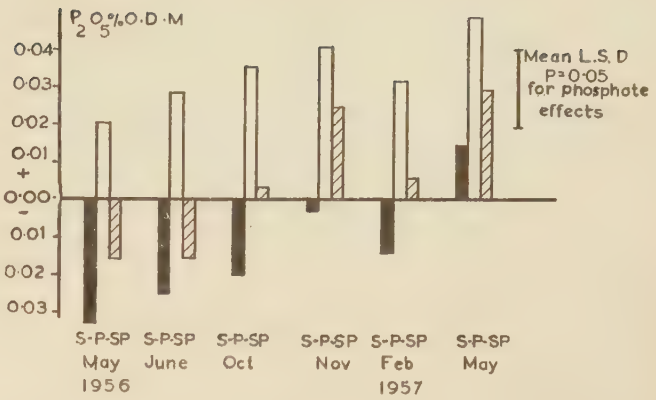

FIGURE 5

Effect of shade (S) and phosphate fertiliser (P) on leaf phosphorus content.

Leaf content and yield

Leaf contents of nitrogen and phosphorus were compared with the yield from the plots sampled. These yields are quoted as total number of ripe pods reaped from the plot (16 trees at the wider spacing and 36 trees at the closer spacing). When comparisons were made with yield of wet cacao per plot the correlations were less marked. This may be due to the fact, already noted (Havord, Maliphant and Cope 1954) that any factor which increases the yield usually decreases the pod content of wet beans.

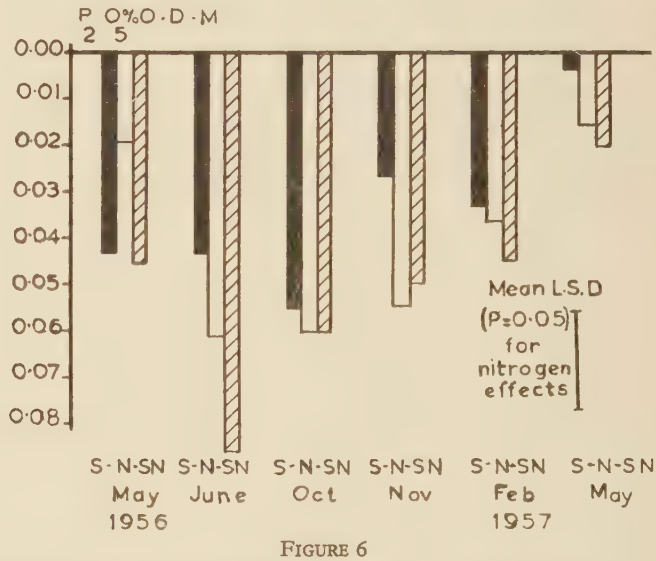

FIGURE 6

Effect of shade (S) and nitrogen fertiliser (N) on leaf phosphorus content.

Nitrogen. Under shaded conditions, there was no significant correlation between leaf nitrogen content and yield. In the absence of shade and in "wide spaced" plots highly significant positive correlations (r=0.66-0.69 d.f. 15) were found between leaf nitrogen content in samples taken in May, June and October, 1956, and the crop reaped September, 1956-August, 1957. Significant correlations (r=0.48 and r=0.49) were also found between samples taken in February or May, 1957, and the crop

reaped September, 1957-August, 1958. A similar state of affairs, with smaller correlation coefficients, existed in samples from " close spaced " plots.

Phosphorus. Leaf phosphorus levels in samples taken in November, 1956, and February, 1957, were negatively correlated with yields (September, 1956,-August, 1957) from unshaded plots. In shaded plots the correlations were highly significant but positive in samples taken in February or May, 1957 (r=0.46 and 0.59 respectively d.f. 31).

Multiple correlations

Unshaded plots. There are two main ways of combining the results noted above :—

(*a*) by multivariate analysis, using results for nitrogen content in samples taken as the main crop was setting (May, June or October, 1956), and results for phosphorus content in samples taken during the cropping period (November, 1956, or February, 1957) and the yields per plot, or

(*b*) by combining nitrogen and phosphorus analyses into one index, the most common one used being the $\frac{N}{P_2O_5}$ ratio.

Using the first method, very high multiple correlation coefficients were found for results from unshaded wide spaced plots combining nitrogen analyses in May, 1956, with phosphorus analyses in November, 1956 (r=0.84 d.f. 15). Correlations from plots with other combinations of shade and spacing were very much poorer. Using the second method it was found possible to combine results from wide and close spaced plots. The $\frac{N}{P_2O_5}$ ratio was significantly correlated with yields reaped September, 1956-August, 1957, for all samples taken after May, 1956. The highest correlation obtained was from samples taken in February, 1957 (Fig. 7) (r=0.59 d.f. 31 : for P=0.001 r=0.55). There was also a highly significant correlation (r=0.48 : for P=0.01 r=0.44) between the $\frac{N}{P_2O_5}$ ratios from samples taken in May, 1956, and the crop reaped September, 1955-August, 1956.

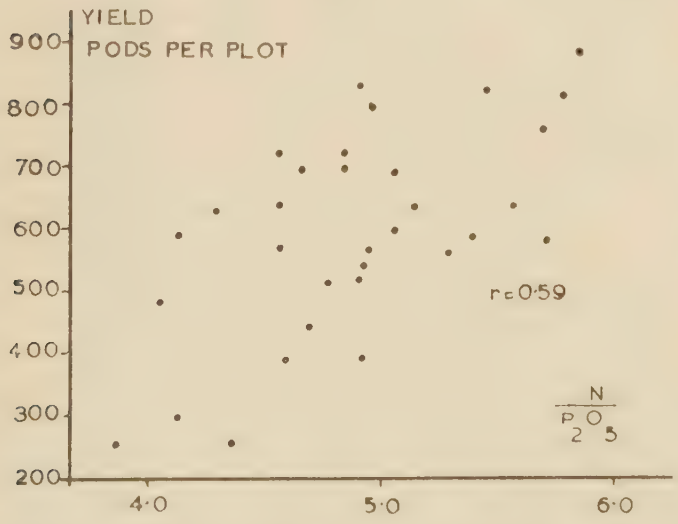

FIGURE 7

Relationship between yield (Sept. 1956-Aug. 1957) and the ratio $\frac{N}{P_2O_5}$ for leaf samples taken from unshaded plots in February, 1957.

Shaded plots. Despite the absence of correlations between yield and leaf nitrogen content from shaded plots, highly significant negative correlations between yield and $\frac{N}{P_2O_5}$ ratio were obtained from samples collected in February (r= −0.47) and May, 1957 (Fig. 8 r= −0.62 d.f. 31 : for P=0.001 r=0.55), and yields reaped September, 1956-August, 1957. No similar correlation was found for the May, 1956, samples and 1955-1956 crops.

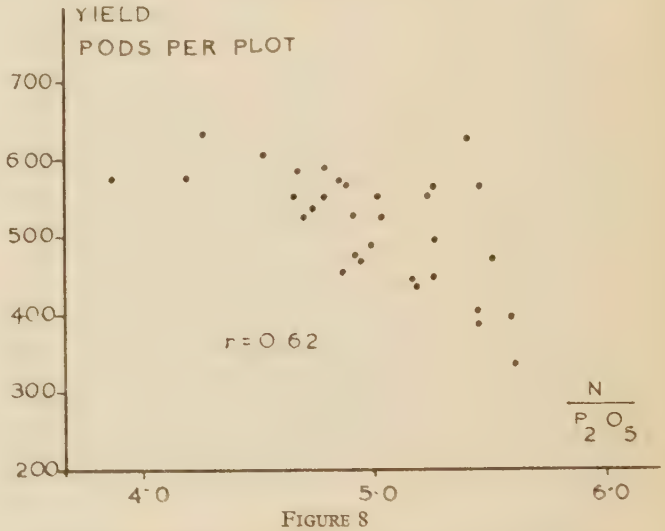

FIGURE 8

Relationship between yield (Sept. 1956-Aug. 1957) and the ratio $\frac{N}{P_2O_5}$ for leaf samples taken from shaded plots in May, 1957.

Discussion

McDonald and Rodriguez (1935) studied the relationship between the composition of leaves and yield at River Estate, using 30 years old seedling cacao growing under shade. They also found that correlations between yields and N, P_2O_5 and $\frac{N}{P_2O_5}$ values were higher when the yields considered were pods per plot and not wet cacao per plot. These workers found a highly significant positive correlation between the $\frac{N}{P_2O_5}$ ratio and yield, and compared their results with samples taken from a " Standard Good Plot " which gave an $\frac{N}{P_2O_5}$ ratio of 4.66.

The apparent contradiction between these results and results presented in this paper fades when it is realised that the range of $\frac{N}{P_2O_5}$ values found by Rodriguez (3.1-3.9) was lower than that shown in Figs. 7 and 8.

The value of the $\frac{N}{P_2O_5}$ ratio in studying the relationship between the chemical composition of the leaf and the yield of cacao is obvious from Figs. 7 and 8, but until more extreme values of this ratio are found no optimum values for this ratio can be given.

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The Cacao Tree Size-Yield Correlation

by

G. K. Maliphant

In previous publications (Jones and Maliphant) it was reported that a highly significant correlation existed between tree girth at $3\frac{1}{2}$ years of age and subsequent yields. Results presented in this paper show the effect of spacing and shade in this correlation and its variation with successive crops.

To illustrate these effects results from Experiment I (Havord, 1953) have been used. Mean girth measurements in February, 1953, of ICS 1 trees planted in July, 1949, have been correlated with yields per plot (48×48 ft.) in kilograms of wet cacao for each crop year from 1952 to 1958. The girths used were the means, in cm., from 16 trees at the wider spacing and 36 trees at the closer spacing, in the presence and in the absence of anauca immortelle (*Erythrina poeppigiana* (Walp.) O. F. Cook) as permanent shade. The shade trees were planted at 24×24 ft. spacing, and thinned later (1956-1957) to 24×48 ft. Results from 18 plots at each combination of shade and spacing were available. The plots have received a variety of fertiliser treatments since 1949, but no other soil amendments or treatments. Table I shows the correlation coefficients so obtained.

Table I

Correlation coefficients (n=18) between girths at $3\frac{1}{2}$ years (1953) and successive crops (kgm. wet cacao per plot) for combinations of shade and spacing

Crop year	1952-3	1953-4	1954-5	1955-6	1956-7	1957-8
Unshaded						
Wide spaced	0.77	0.82	0.79	0.79	0.74	0.42
Close spaced	0.63	0.39	0.43	N.S.	N.S.	N.S.
Shaded						
Wide spaced	0.84	0.85	0.87	0.75	0.38	0.44
Close spaced	0.54	N.S.	N.S.	N.S.	N.S.	N.S.

L.S.D. (P=0.05) 0.46. N.S. non-significant correlation.

Table I shows that the highly significant correlation previously noted for cacao planted 12×12 ft. under immortelle shade is also found in results from unshaded plots. It is remarkable that after several years of significant correlations these disappeared after the eighth year in the case of unshaded cacao and after the seventh year in shaded cacao. From close spaced trees (8×8 ft.) significant correlations were only found in the first crop.

If factors affecting the early growth of cacao trees are kept at or near an optimum, high yields will be obtained during the early years of cropping. If these factors are not carefully controlled much lower yields will be obtained in the early years but, by about the eighth year in the case of wide spaced trees, the yields will rise to something approaching the yields of carefully tended plots in later years. The practice of methods to control such factors as are operating will, therefore, depend on the cost of such methods and the value of the increased crop produced.

The graph previously published shows that if methods can be devised to produce trees of 30 cm. or more in girth by $3\frac{1}{2}$ years the increase in yield from wide space plots under the conditions of the experiment, would be of the order of 400-600 lb. dry cacao per acre more than trees of 20 cm. in girth. It has already been suggested that two factors which might be of great importance in affecting the size of trees are weed competition and soil moisture deficits.

It is a well-known fact that when cacao trees are close planted, say 8×8 ft., then competition for nutrients and soil moisture from weeds, amongst which must be included the banana traditionally used as temporary shade for cacao, is rapidly eliminated by the self shading effect of the cacao canopy. Weed growth in wide space plots continues for many years after it has been virtually stopped in close spaced plots.

It is probable, therefore, that one of the simplest and most effective means of producing high yielding plots of cacao is to plant the trees close together. As is illustrated elsewhere in this Report, the yield per tree would be much lower, but the yield per acre higher than if the trees were planted wider apart. After nine or ten years the plantation could be progressively thinned.

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Manurial and Cultural Experiments on Cacao— Part VI. River Estate, Trinidad

by

G. K. Maliphant

Most of the manurial and cultural field experiments at River Estate have been described in previous reports for 1945/51, 1952, 1953, 1954 and 1955/56 by Havord, Maliphant and Cope. This paper describes additional experiments, and notes on experiments abandoned.

New experiments

XI. Chlorosis experiment

The experiment originally designed as a Trace Element Experiment (Havord, Report for 1952, pp. 44-46) was redesigned and planted at San Pablo Estate, Gran Couva, Trinidad, in 1956. During soil surveying work in the area of Gran Couva, which will be published elsewhere, Havord noted small flat areas, usually in the bends of the river, where the soil had a high content of free calcium carbonate throughout the profile (up to 30% $CaCO_3$ in the surface). These areas were usually delineated by the presence of a small plant identified as *Pilea ovalis*. It is in such areas that lime-induced chlorosis, sometimes referred to as iron chlorosis, is experienced.

The treatments incorporated in the experiment are :—

(*a*) Shade. Two blocks are shaded, mainly by a large silk cotton tree. There are a few immortelle trees present. Two other blocks are unshaded.

(*b*) Four soil treatments :

(i) control ;

(ii) iron applied as ferric ammonium citrate 4 oz. per tree ;

(iii) iron applied as hydroxy EDTA complex (2 levels of application 1 oz. and 4 oz. per tree) ;

(iv) sawdust mulch—12 tons per acre.

(*c*) Foliar spray. There is accommodation in the design for a foliar spray of ferrous sulphate to be applied as required.

(*d*) Clones. Four clones are used—ICS 1, ICS 60, ICS 89 and ICS 95.

The sawdust mulch treatment has been included because of considerable success in the control of chlorosis in pot culture of cacao (Havord, Report for 1954, pp. 72-76). A supply of the hydroxy-EDTA complex was kindly supplied by the Dow Chemical Company.

With the exception of the foliar sprays, treatments, along with a general fertiliser application were completed in November, 1958. Regular measurements of growth and chlorotic symptoms will be made as well as subsequent yields.

XII. Shade, chupon, fan, seedling experiment

Some observations on the need for this experiment have already been given (Havord, Report for 1952, pp. 44-46). The experiment was planted in 1953 and incorporated the following treatments :—

(*a*) Shade *versus* no shade. The shade tree used is the Anauca immortelle (*Erythrina poeppigiana* (Walp.), O. F. Cook), planted 24×24 ft.

(*b*) Spacing. Two spacings, 12× 12 ft and 8×8 ft. are used.

(*c*) Planting material :

(i) fan cuttings, with chupons removed ;

(ii) fan cuttings, with one chupon allowed to establish itself and eventually eliminate the fan material ;

(iii) mixed seedlings, from known high bearing clonal parentage.

Results

Two crops have been reaped and recorded—1956-1957 and 1957-1958, the mean yields being 171 and 589 lb. dry cacao per acre respectively.

As would be expected from the results of Experiment I (Havord, Maliphant and Cope, Reports for 1953, pp. 80-87 and for 1954, pp. 58-64) the effect of shade was very small, being slightly larger on close spaced cacao (Table I). Similarly, close spaced trees outyielded wide spaced trees in yield per acre, but the reverse is true in yield per tree.

Table I

Yield in lb. dry cacao per acre (1957-1958 crop)

Type of planting Material		Fan cutting		Selected Seedlings	Means
		No chupon	One chupon allowed		
Closed spaced	No shade	1,120	641	262	674
	Shade	1,250	903	295	816
	Mean	1,185	772	278	745
Wide spaced	No shade	727	339	182	416
	Shade	595	543	208	449
	Mean	661	441	195	433
Means		923	606	237	589

In these two crops fan material highly significantly outyielded chupon trees. Seedling trees gave very poor yields when compared with the clonal plants.

Three plots each with 36 trees yielded at a rate of over 1,000 lb. dry cacao per acre in their fifth year.

XIII. Mulching experiment

The value of mulches, either of cut grasses or pen manure is well-known, and well borne out by the results of experiments at River Estate. The use of low grade (i.e. low NPK content) mulch, e.g. bagasse, which decomposes slowly, but gives improved structure to the topsoil, has not proved to be an outstanding success. In all the experiments so far some attempt has been made to maintain chemical equivalence by adding chemical fertiliser after analysis of samples of mulch.

In view of the results from other experiments, this experiment was designed to study the effect of two types of mulch untrammelled by other " non-mulch " treatments, with and without chemical fertiliser. The low grade mulch selected was sawdust, from a mixture of woods and obtained from a local saw mill. Bagasse has been used in another experiment, but is not readily available in non-sugar-growing areas.

The experiment was planted in 1954, and the following treatments commenced in September, 1956 :—

(i) Sawdust mulch—12 tons per acre, giving a layer of approximately $\frac{1}{2}$ in., applied in September of each year.

(ii) Bush mulch (i.e. mixture of grasses, weeds with some woody weeds as grown on waste areas in the estate)—12 tons per acre, applied in September, 1956, and subsequently May and September each year.

(iii) Bush mulch as (ii) applied whenever required at 12 tons per acre to maintain a mulch cover all through the year. It is expected that 3 or 4 applications per year will be needed.

(iv) Factorially combined NPK treatments—superimposed on the mulch treatments, as ammonium sulphate, double superphosphate and potassium chloride at levels determined from the results of Experiment I (Havord, Report for 1945-1951, pp. 104-108). As from September, 1959, these applications will be made as a split dose, half in May and half in September.

The experiment is designed as a single replicate of a factorial 2^5, with certain high order interactions confounded with block differences. Each plot contains 30 trees, one each of 30 different clones, spaced at 12×12 ft. Mulch applications are mechanised as far as practicable. 30 clones were used to give a mixed population for flavour tests.

The first recorded crop (mean yield 102 lb. dry cacao per acre) was reaped in 1957-1958. Although no significant results were obtained plots receiving the " luxury " mulch application (iii above) yielded considerably more cacao than other plots.

Abandoned experiments

The following experiments previously described (Havord, Reports for 1945-1951, pp. 104-108 and for 1952, pp. 44-46) have been abandoned.

V. planting hole size experiment

After 7 years no regular trend in results has appeared. It might be expected that the largest planting holes would enable the plants to become well-established in a nutrient rich medium. However, as the pen manure decomposed the soil in the immediate vicinity of the plant sank so forming a small basin. The soil being only imperfectly drained, this enabled water to collect around the trees so offsetting any ameliorating effect of the larger quantities of pen manure by incipient waterlogging.

VI. Micro-plot experiment

This experiment in its early stages afforded proof of the increased aeration and soil-aggregate stability resulting from mulch applications. The trees in the experiment have subsequently been used for studies in root distribution and spread.

VII. Gliricidia mulching experiment

The soil on which this experiment was planted is classed by Chenery as Las Lomas Fine Sand, an infertile soil with very little water-retention properties. During at least two dry seasons a large amount of supplementary watering was necessary. Considerable difficulty was experienced in establishing gliricidia over the whole area. After supplying cacao plants, where necessary, in the initial three years, it was realised that if the experiment was to be fully supplied the resulting age range of the cacao would be very broad. At the same time, it was discovered that in several parts of the experiment the cacao (ICS 95) was withstanding the adverse conditions better than the gliricidia.

X. Root growth experiment

It was found impossible to obtain the correct number of grafted trees using ICS 62 as the " poor " clone. In view of the volume of results coming from the other experiments at River Estate and elsewhere, it was felt ill-advised to re-plan this experiment.

Summary

Details of new experiments planted and old experiments which have been abandoned are given.

Manurial and Cultural Experiments on Cacao. Part VII

River Estate, Trinidad

Shade, Spacing, Times and Rates of Fertiliser Application Experiment

by

G. K. Maliphant

SOME early results from this experiment have already been published (Havord, Maliphant and Cope 1954, 1955). This paper presents results up to the crop year 1957-1958 from the experimental plots and the guard plots.

Experimental plots

Table I gives the yields up to the crop year 1957-1958, excluding those already published for the first two crops. The mean yields for the successive crops were 1,028, 1,022, 1,198 and 1,130 lb. dry cacao per acre. The rates of fertiliser applications were N, 450 lb. ammonium sulphate, P, 150 lb. double superphosphate, and K, 300 lb. potassium chloride per acre. All fertiliser applications were in May.

Shade. With the exception of the effect on close spaced plots in the 1957-1958 crop, shade reduced yields. The overall shade effect has never attained statistical significance.

Spacing. Under shaded conditions, close spaced plots highly significantly outyielded wide spaced plots (mean effect over 4 crops 175 lb. dry cacao per acre). In the absence of shade, the same effect was more marked in the first 3 crops (mean effect 239 lb. dry cacao per acre), but in the most recent crop wide spaced unshaded plots gave 104 lb, more cacao per acre than close spaced unshaded plots. This effect was due to a very large drop in yield from all but two of the close spaced unshaded plots. At all time wide spaced trees yield much more cacao per tree than closed spaced trees.

TABLE I

Yields in pounds of dry cacao per acre for combinations of shade, spacing and fertilisers.

		Crop year	O	N	P	NP	K	NK	PK	NPK	Means
Close spacing	No shade	1954-55	1,252	1,351	1,050	1,349	717	1,453	1,131	1,309	1,202
		1955-56	1,066	1,283	1,220	1,457	987	1,356	1,268	1,390	1,253
		1956-57	1,125	1,286	1,341	1,640	926	1,503	1,521	1,522	1,358
		1957-58	634	1,321	1,022	1,149	593	1,085	1,000	1,586	1,048
		Means	1,019	1,310	1,158	1,399	806	1,349	1,230	1,452	1,215
	Shade	1954-55	1,080	1,069	1,135	1,071	1,035	1,117	1,091	1,173	1,096
		1955-56	1,041	1,039	1,001	1,074	954	943	1,106	1,162	1,040
		1956-57	1,147	1,225	1,241	1,306	1,085	1,094	1,282	1,113	1,187
		1957-58	1,045	1,227	1,240	1,267	1,134	1,092	1,416	1,391	1,226
		Means	1,078	1,140	1,154	1,179	1,052	1,061	1,224	1,210	1,137
	Means		1,049	1,225	1,156	1,289	929	1,205	1,227	1,331	1,176
Wide spacing	No shade	1954-55	185	1,314	993	1,210	618	1,362	680	1,108	934
		1955-56	424	1,174	1,147	1,210	697	1,356	832	1,043	985
		1956-57	899	1,225	1,082	1,403	923	1,573	1,010	1,304	1,177
		1957-58	830	1,023	938	1,471	1,017	1,430	1,194	1,312	1,152
		Means	585	1,184	1,040	1,324	814	1,430	929	1,192	1,062
	Shade	1954-55	718	905	960	990	904	832	760	972	880
		1955-56	651	788	850	810	765	958	897	750	809
		1956-57	956	996	1,192	957	1,122	1,092	1,106	1,128	1,069
		1957-58	882	1,145	1,090	1,142	960	1,180	1,296	1,039	1,092
		Means	802	958	1,023	975	938	1,016	1,015	972	962
	Means		693	1,071	1,032	1,149	876	1,223	972	1,082	1,012
Means			871	1,148	1,094	1,219	902	1,214	1,099	1,207	1,094

TABLE II

Mean yields (1954-1958) in pounds of dry cacao per acre for combinations of shade and spacing, rates, R, and time, T, of application of fertiliser.

		O	N	P	NP	K	NK	PK	NPK	Means
Close spaced unshaded plots	R_2T_1	1,310	1,179	1,290	1,399	1,083	1,206	1,273	1,526	1,283
	R_2T_2	1,081	1,447	1,192	1,522	1,119	1,358	1,304	1,651	1,334
	R_3T_1	1,237	953	1,010	1,548	859	1,205	1,095	1,454	1,170
	R_3T_2	1,138	1,259	1,372	1,386	1,318	1,273	1,347	1,460	1,319
	Means	1,192	1,210	1,216	1,464	1,095	1,261	1,255	1,523	1,277
Wide spaced shaded plots	R_2T_1	849	1,026	1,081	997	1,005	966	850	1,076	981
	R_2T_2	910	939	1,190	1,049	1,055	971	1,300	1,227	1,080
	R_3T_1	1,047	902	952	1,097	973	1,028	918	1,159	1,010
	R_3T_2	890	964	921	1,082	873	1,097	1,268	1,083	1,022
	Means	924	958	1,036	1,056	977	1,015	1,084	1,136	1,023
Means		1,058	1,084	1,126	1,260	1,036	1,138	1,169	1,330	1,150

Nitrogen fertiliser (applied as ammonium sulphate). The nitrogen effect was only significant in the absence of shade and was highest in wide spaced plots (mean response 440 lb. dry cacao per acre). Close spaced plots receiving nitrogen fertiliser yielded 324 lb. dry cacao per acre more than plots not receiving nitrogen fertiliser. The drop in yield from unshaded close spaced plots noted above was smaller from plots receiving nitrogen fertiliser. The effect of nitrogen fertiliser on shaded cacao was negligible. The mean increases in yield from nitrogen fertilisers was 36 lb. dry cacao per acre in wide spaced plots and 11 lb. dry cacao per acre in close spaced plots.

Phosphate fertiliser (applied as double superphosphate). The increase in yield from phosphate fertiliser was, in general, only significant in the absence of nitrogen fertiliser and higher in unshaded plots. In the presence of nitrogen fertilisers, phosphate fertiliser tended to reduce yields in wide spaced plots. In close spaced plots increases were small.

Potash fertiliser (applied as potassium chloride). The effect of potash fertiliser has been variable and small, but with a tendency to increase as the experiment aged.

Guard plots

From September, 1954, the 128 guard plots in this experiment were used to investigate the effect of different rates and times of application of fertilisers. The plots were divided into blocks for the comparison of two rates of application, twice and three times that used in the experimental plots, two times of application of fertiliser, either all in May or half in May and half in September, and two combinations of shade and spacing, viz. shaded wide spaced cacao and unshaded close spaced cacao. There were two replicates. Within the blocks fertilisers were factorially applied as in the experimental plots. For analysis purposes certain high order interactions between the treatments were confounded between the 32 sub-blocks of 4 plots used.

Table II gives the mean yields over the 4 crops under discussion from combinations of the above treatments. R_2 indicates fertilisers applied at 900 lb. ammonium sulphate, 300 lb. double superphosphate, 600 lb. potassium chloride per acre. R_3 indicates applications at $1\frac{1}{2}$ times R_2. T_1 indicates applications in May and T_2 applications in May and September.

TABLE III

Mean yields in pounds of dry cacao per acre for combinations of shade and spacing, rates, R, and times, T, of fertiliser applications for four successive crops.

	Crop year	1954-55	1955-56	1956-57	1957-58	Means
Close spaced unshaded plots	R_2T_1	1,217	1,171	1,429	1,316	1,283
	R_2T_2	1,274	1,251	1,499	1,312	1,334
	R_3T_1	1,029	1,164	1,304	1,184	1,170
	R_3T_2	1,255	1,228	1,444	1,348	1,319
	Means	1,194	1,204	1,419	1,290	1,277
Wide spaced shaded plots	R_2T_1	870	820	1,075	1,161	981
	R_2T_2	1,013	847	1,213	1,247	1,080
	R_3T_1	871	926	1,041	1,201	1,010
	R_3T_2	969	846	1,122	1,152	1,022
	Means	931	860	1,113	1,190	1,023
Means		1,062	1,032	1,266	1,240	1,150

The mean yields for the 4 crops for combinations of shade and spacing, rates and times of fertiliser application are given in Table III.

Thirteen plots, one of them wide spaced and shaded, yielded over 1,700 lb. dry cacao per acre in 1956-1957 or/and 1957-1958 crops.

Shade and spacing. As would be expected from results quoted earlier for the experimental plots, close spaced unshaded plots yielded much more cacao per acre than wide spaced shaded plots.

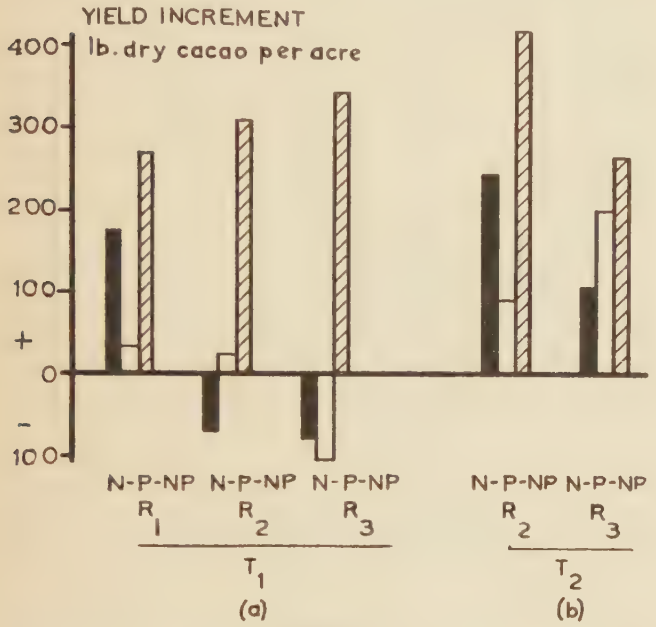

FIGURE 1

Responses to nitrogen fertiliser (N) and phosphate fertiliser (P) in close spaced unshaded plots.

Rates and times of fertiliser application (Table III). The overall effect of the rates and times of application shows that highest yields were obtained from a split application of fertiliser (T_2) at the rate, R_2, of 900 lb. ammonium sulphate, 300 lb. double superphosphate and 600 lb. potassium chloride per acre.

Fertilisers. The effect of fertilisers and interactions with rates and times of application are shown in Figs. 1, 2, 3 and 4, where the differences in yield between the fertilised plots and the mean of all the 10 unfertilised plots in the experiment for the 4 crops are plotted. The mean yields of the unfertilised plots were 1,157 lb. of dry cacao per acre from close spaced unshaded plots and 900 lb. of dry cacao per acre from wide spaced shaded plots. Results from the experimental plots are incorporated in these figures as R_1T_1 applications.

Close spaced unshaded plots

The response to nitrogen fertilisers when applied all at once (T_1) was much higher in the presence of phosphate fertilisers. This interaction was largest at the highest rate of fertiliser application (R_3) and was apparently increasing with successive crops. It is probable that using high rates of nitrogen or phosphate fertiliser dressing alone caused an acute imbalance in the nutrition of the tree resulting in a depression of yields at the highest rate, R_3. When that imbalance was at least partially corrected by using phosphate and nitrogen fertilisers together large positive responses were obtained (Fig. 1 (a.))

When fertilisers were applied as a split dressing, Fig. 1 (b) (only results from rates of application R_2 and R_3 are available) responses to nitrogen fertiliser were much higher than when applied all at one time. However, the response was larger at the lower rate. The interaction between nitrogen and phosphate fertilisers was much less than in the previous case and was decreasing with successive crops.

It is unfortunate that the experiment could not incorporate a treatment of fertilisers applied as a split dressing at the lowest rate, R_1. The largest response to nitrogen fertiliser has been when the fertiliser was applied as a split dressing at the intermediate rate, R_2. The experimental plots are to receive fertilisers as a split dressing as from September, 1958, to discover whether the response at the lowest level R_1 will approach or even exceed that at higher levels.

Fig. 2 (a) shows the responses to potassium fertiliser and their interaction with nitrogen fertiliser when applied all at one time. As with phosphate fertilisers, when applied without nitrogen fertiliser the responses to potash fertilisers passed through a maximum at or near rate R_2. In the presence of nitrogen fertiliser responses to potash fertiliser applications increased with the higher rates of application and also increased with successive crops, especially at the highest rate of application R_3.

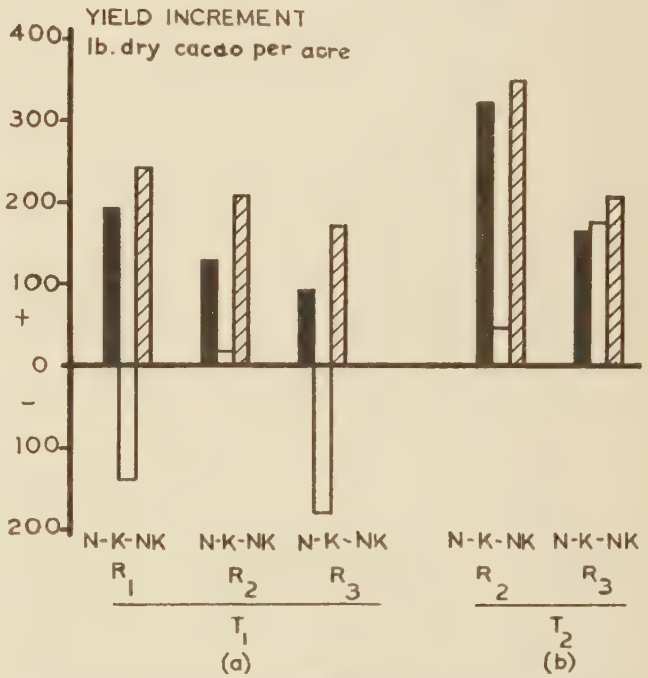

FIGURE 2

Responses to nitrogen fertiliser (N) and potash fertiliser (K) in closed spaced unshaded plots.

When fertilisers were applied as a split dressing, Fig. 2 (b), again similar to the results from phosphate fertilisers, responses from potash fertilisers were positive with a negative interaction with nitrogen fertilisers.

The overall response to potash fertilisers in the first three crops has been negative or only small and positive, but in

the fourth crop an extremely large response to potash fertilisers was obtained. This crop was also notable in that it was reaped mainly in the second half of the crop year, whereas previous crops have been picked in the first half of the year. The causes for this abrupt change of cropping are still being sought.

Wide spaced shaded plots

Figs. 3 and 4 are the histograms for wide spaced shaded plots corresponding with Figs. 1 and 2.

Responses to nitrogen fertiliser were much lower under shaded conditions and the effect of increasing the rate was much less marked. When applied as a single dressing each year (Fig. 3 (a)) the response to nitrogen or phosphate fertilisers alone decreased as the rate was increased but, as in unshaded cacao, when applied together (NP) the response increased considerably as the rate was increased. The overall effect of phosphate fertilisers has increased, particularly at the higher rates of application, over the 4 crops considered.

When fertilisers were applied as a split dressing (Fig. 3 (b)) the effect of nitrogen fertiliser was higher at the R_3 rate and the effect of phosphate fertiliser was higher at the R_2 rate. The interaction between these fertilisers was large and negative at both rates of application. Again the effect of phosphate fertilisers has increased with successive crops.

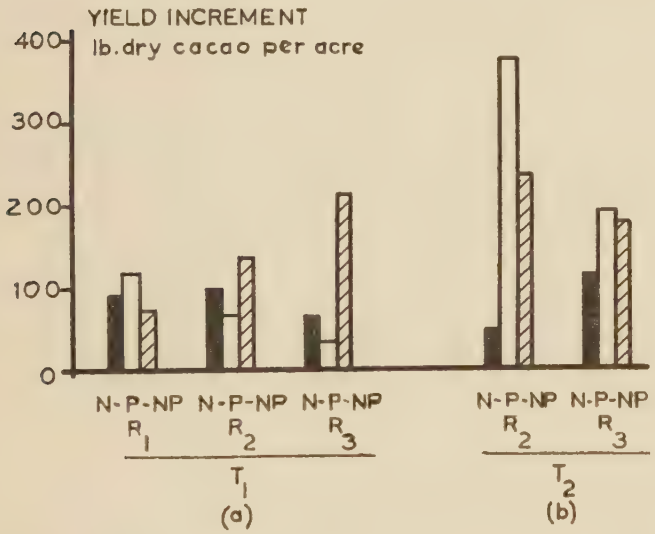

FIGURE 3
Responses to nitrogen fertiliser (N) and phosphate fertiliser (P) in wide spaced shaded plots.

The effect of potash fertilisers and its interaction with nitrogen fertiliser is shown in Fig. 4. Responses to potash fertiliser were higher under shaded conditions than under unshaded conditions, particularly when applied as a split dressing. Responses to potash fertiliser were usually lower in the presence of nitrogen fertiliser.

Summary

The effect of fertilisers, rates and times of application, shade and spacing on yields of cacao are discussed. Responses to nitrogen fertiliser were higher in the absence of shade and highest when applied as a split dressing (50% in May, 50% in September) at 900 lb. ammonium sulphate per acre in the presence of phosphate fertiliser, 300 lb. double superphosphate per acre.

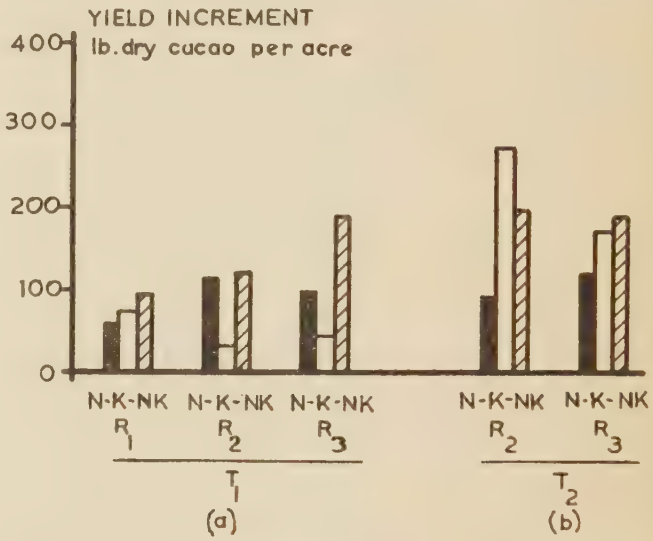

FIGURE 4
Responses to nitrogen fertiliser (N) and potash fertiliser (K) in wide spaced plots.

Responses to phosphate fertiliser were generally higher in the absence of shade, but were highest when applied as a split dressing at 300 lb. double superphosphate per acre under shaded conditions. Responses to potash fertiliser were highest in the presence of shade when the fertiliser was applied as a split dressing of 600 lb. potassium chloride per acre. The effects of phosphate and potash fertiliser increased in successive crops.

As there were no areas receiving fertilisers at the lowest rate as a split dressing, this is being applied on the experimental plots as from September, 1958, as it is possible that the optimum dressing under existing conditions of the experiment is at or near this rate of application of fertiliser.

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